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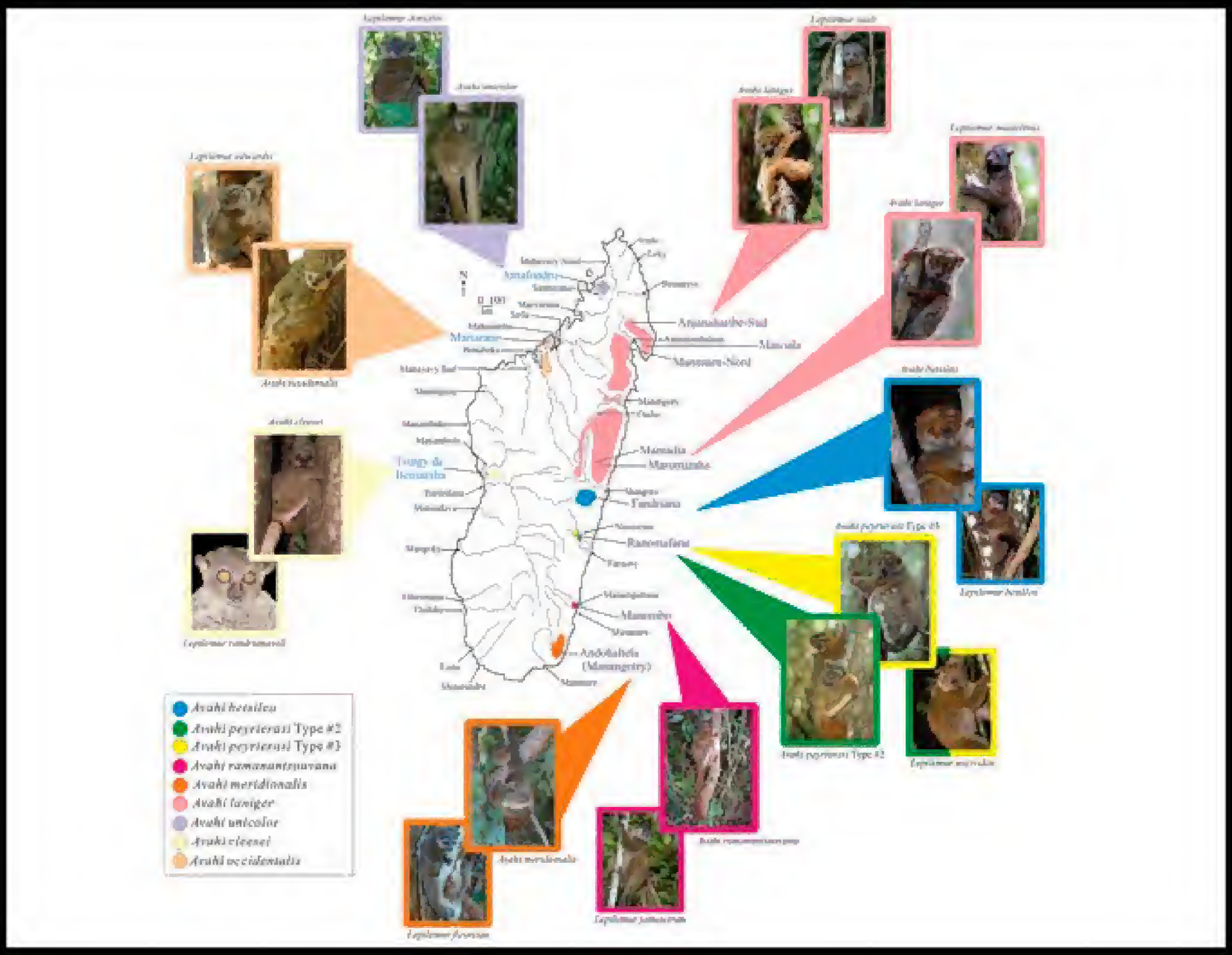
Molecular Phylogeny and Taxonomic Revision of the Woolly Lemurs, Genus *Avahi* (Primates: Lemuriformes)



Rambinintsoa Andriantompohavana, et al.

Front cover: Revised distribution of the woolly lemurs of Madagascar. The revisions of the sportive lemurs (Louis et al. 2006b) and this publication demonstrate the partitioning of unique *Avahi* and *Lepilemur* species by rivers which act as barriers. Each region is defined by a distinct woolly and sportive lemur. Figure created by Kelly Herrington and Edward E. Louis, Jr.

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RAMBININTSOA ANDRIANTOMPOHAVANA, RUNHUA LEI, JOHN R. ZAONARIVELO, SHANNON E. ENGBERG, GÉRARD NALANIRINA, SUSIE M. MCGUIRE, GARY D. SHORE, JUSTIN ANDRIANASOLO, KELLY HERRINGTON, RICK A. BRENNEMAN, AND EDWARD E. LOUIS, JR.

University of Antananarivo and Henry Doorly Zoo

Layout and Design: Jacqueline Chavez
Cover Design: Kelly Herrington and Edward E. Louis, Jr.

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Museum of Texas Tech University
Lubbock, TX 79409-3191 USA
(806)742-2442

Molecular Phylogeny and Taxonomic Revision of the Woolly Lemurs, Genus *Avahi* (Primates: Lemuriformes)

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Abstract

Approximately 3,000 base pairs of mitochondrial DNA sequence data (control region or D-loop, along with a fragment including a partial segment of cytochrome oxidase subunit III gene to Leucine-tRNA gene), morphological and phenotypic characters were used to investigate the phylogenetic relationships among the recognized woolly lemurs (genus *Avahi*) and to other lemur genera of Madagascar. The molecular data support the previously recognized *Avahi* species, but it has also revealed additional unique biodiversity. Here, we present further taxonomic revisions of the genus *Avahi*, by elevating the two recently described subspecies, *A. m. meridionalis* and *A. m. ramanantsoavana*, to species level, along with a description of one new species. We also conducted the first phylogenetic analysis of all recognized species of woolly lemurs, all representatives of the family Indriidae, and present the relationships between the recognized species of the genus *Avahi* and this newly described species. These results underscore the urgency to initiate further detailed studies in previously unstudied sites throughout Madagascar in order to better define lemur species.

Key words: *Avahi*, D-loop, Madagascar, prosimian, systematic, woolly lemurs

Introduction

Due to its unique species biodiversity and the continued pressure from human encroachment in the form of habitat destruction and fauna and flora utilization, Madagascar has been placed at the top of conservation priority lists, or hotspots (Myers 2000). Distributed throughout the island, the prosimians of Madagascar are particularly susceptible to extinction risks due to their relatively small, fragmented geographic ranges, and the potential loss through edge effects and constant habitat loss (Jernvall and Wright 1998; Reed 2004; Ezard and Travis 2006). Since 2000, multiple molecular genetic and morphological studies within the genera *Avahi*, *Cheirogaleus*, *Lepilemur*, *Microcebus*, and *Mirza* have led to a significant increase in the number of recognized species (Zimmermann et al. 1998; Groves 2000; Rasoloarison et al. 2000; Kappeler et al. 2005; Thalmann and Geissmann 2005; Andriaholinirina et al. 2006; Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b; Zaramody et al. 2006). With the addition of newly described species, the once broad ranges of the previously recognized nocturnal species have dramatically decreased and concurrently made the risk of extinction of both recognized and newly described lemurs much greater (a smaller distribution and a diminished number of represented subpopulations). Currently, all lemurs are protected under the Convention of International Trade of Endangered Species (CITES) and are designated by the IUCN/SSC Red List Categories from critically endangered to threatened (IUCN 2004). Recent revisions underscore the need to resolve the taxonomy and phylogeny of lemurs so that a scientifically rational approach to their conservation and management can be developed and implemented. Furthermore, the numerous taxonomic revisions of lemurs have led to subsequent modifications of historical distributions and population estimates that require a persistent re-evaluation of conservation protection status (Martin 2000; Louis et al. 2006b).

The woolly lemurs (genus *Avahi*), a group of strictly nocturnal primates that are regarded as phenotypically indistinct with varying degrees of brown, red, white, and gray pelage, are a prime example of species whose taxonomic/evolutionary diversity until now has been largely unrecognized and underrepresented. Traditionally, two species of *Avahi* have been recognized, one from eastern Madagascar, *Avahi laniger*, and one from western Madagascar, *Avahi occidentalis* (Fig. 1). Initially, both species were considered subspecies of *Avahi laniger*, but morphological, molecular, and cytogenetic differences between the eastern woolly lemur, *Avahi laniger*, and the western woolly lemur, *Avahi occidentalis*, have conclusively established species level variance (Rumpler et al. 1990; Thalmann and Geissmann 2000; Zaramody et al. 2006; Figs. 2-4).

The potential for additional species within the genus *Avahi* was predictable, given the discrete and often fragmented distributions, the scattered or spotty occurrence within these fragments, and the widespread area that the genus *Avahi* is found especially on the eastern coast of Madagascar (Martin 2000; Thalmann and Geissmann 2000). A comprehensive study to define these populations is critical above and beyond the threat of habitat loss because the highly folivorous dietary requirements of the woolly lemur can bring about a selective liability that can potentially limit habitat suitability. Consequently, Thalmann and Geissmann (2000, 2005) based on morphological and vocalization data described two additional woolly lemurs from western Madagascar, *A. unicolor* and *A. cleesei*, from the Ampasindava region and Tsingy de Bemaraha, respectively (Figs. 1, 5-7). Furthermore, Zaramody et al. (2006) described two new species from the southeastern coast, *A. peyrierasi* (south of the Mangoro/Onive Rivers to Ranomafana National Park) and *A. meridionalis* (Manombo Special Reserve to Andohahela National Park; Fig. 1). *Avahi meridionalis* was further subdivided into two subspecies, *A. m. meridionalis* and *A. m. ramanantsoavana*, from Sainte Luce/Andohahela National Park and Manombo Special Reserve, respectively (Fig. 1). Here, we present further taxonomic revisions of the genus *Avahi*, along with a description of one new species. We also conducted the first phylogenetic analysis of all recognized species of woolly lemurs, all representatives of the family Indriidae, and present the relationships between the recognized species of the genus *Avahi* and this newly described species.

As previously discussed in Andriantompohavana et al. (2006), Louis et al. (2006a, 2006b), and Thalmann and Geissmann (2005), the utilization of whole vouchers as the designated holotype for a new species is not a prerequisite, but that opportunistic collections can later supplement morphological, vocalization, and/or molecular data in combination with curated blood and/or tissue samples. The woolly lemurs are a prime candidate for this methodology because the highly folivorous dietary requirements of this group of lemurs currently precludes any attempts to curate "live vouchers" (Thalmann and Geissmann 2005). Total genomic DNA for the three paratype specimens are currently curated at the Museum of Texas Tech University (TK125757; TK125758; TK125759). Additionally, an electronic database that includes all *Avahi* field data and photographs, including data for the paratype specimens, is curated at the Museum of Texas Tech University. The database is stored in the Type Specimen Collection in multiple media formats. This collection of field data and photographs, as well as additional tables and figures, also are available online at the website of Omaha's Henry Doorly Zoo. See Appendix I for a directory of appropriate website addresses.

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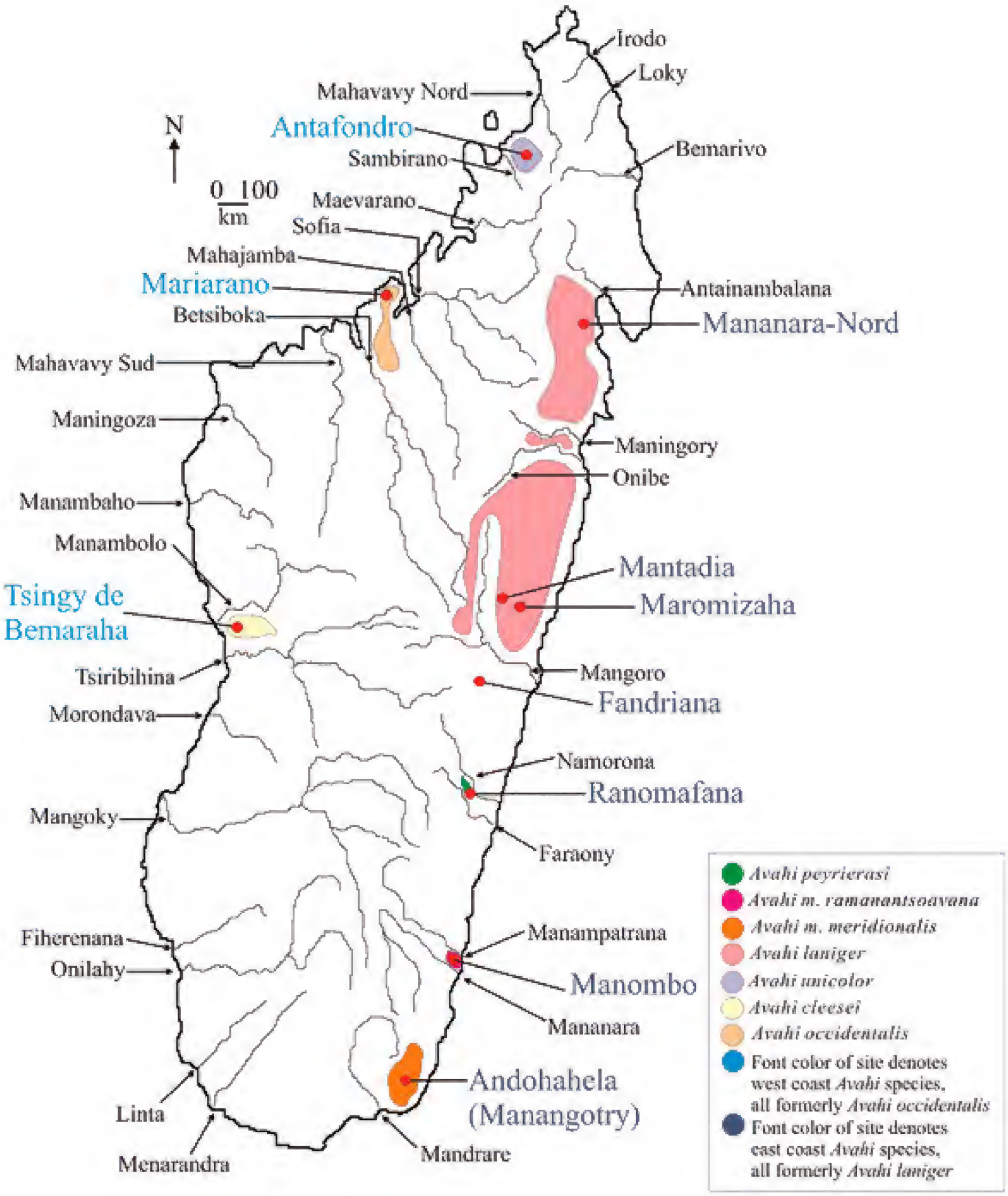

Figure 1. Sample distribution map of the woolly lemurs of Madagascar.

Figure 2. *Avahi occidentalis*, western woolly lemur, at Mariarano Classified Forest. Photo by Edward E. Louis, Jr.

Figure 3. *Avahi occidentalis*, western woolly lemur, at Mariarano Classified Forest. Photo by Edward E. Louis, Jr.

Figure 4. *Avahi laniger*, western woolly lemur, at Mananara-Nord National Park (Ivontaka-Sud). Photo by Edward E. Louis, Jr.

Figure 5. *Avahi unicolor*, Sambirano woolly lemur, at Antofondro Classified Forest (Maromiandra). Photo by Edward E. Louis, Jr.

Figure 6. *Avahi unicolor*, Sambirano woolly lemur, at Antofondro Classified Forest (Maromiandra). Photo by Rambinintsoa Andriantompohavana.

Figure 7. *Avahi cleesei*, Cleese's woolly lemur, at Tsingy de Bemaraha National Park. Reprinted with permission of the author and journal (Thalmann and Geissmann 2006). Photo by Urs Thalmann.

METHODS

Sampling.—All lemurs investigated in this study were wild-caught (sampling localities are depicted in Fig. 1) and were immobilized with a CO_2 projection rifle or blowgun with 10 mg/kg of Telazol (Fort Dodge Animal Health; Overland Park, Kansas). Four 2.0 mm biopsies and 1.0 cc per kilogram of whole blood were collected from each sedated animal and immediately stored in a room temperature storage buffer (Longmire et al. 1992). The location of the immobilized lemur was recorded using a global positioning system (Appendix Ia). Genomic DNA was extracted from a 2.0 mm ear punch using a phenol-chloroform extraction (Sambrook et al. 1989; Appendix II). Beginning in 2000, while the animal was sedated, we placed a HomeAgain microchip (Schering-Plough Veterinary Corp.; Kenilworth, New Jersey) subcutaneously between the scapulae of each lemur (Appendix II). This procedure was used to field catalog each animal with a unique recognition code to provide for the capability to positively re-identify all captured individuals during any future immobilizations. Measurements were taken on the sedated animals as follows: weight (+/- 0.01 kilograms); head crown ((total length from tip of the nose (not the soft tissue) to the occipital crown) +/-0.1 cm); body length ((total length of body from the occipital crown of the head to the base of tail) +/-0.1 cm); tail length ((total length from base of tail to the end of the last caudal vertebra) +/-0.1 cm); forelimb measurements (thumb (total length of the thumb (proximal, middle, and distal phalanges) from the distal tip to proximal point of the last bone of the thumb (claw not included) +/-0.1 cm), longest digit (total length of the phalange only (proximal, middle, and distal phalanx, claw and metacarpal not included) +/-0.1 cm), and hand (total length of hand (phalanges and metacarpals included to the carpal joint) +/-0.1 cm)); radius/ulna ((carpal joint (styloid process of the radius) to the olecarnon tip of the ulna) +/-0.1 cm); humerus ((greater tubercle of humerus to the end of the lateral condyle of the humerus) +/-0.1 cm); hindlimb measurements ((toe (total length of the toe (proximal, middle, and distal phalanges not including claw) from tip to insertion point of the last bone of the hind toe (1st digit) +/-0.1 cm), longest digit (total length of the phalange only (claw and metatarsal not included) +/-0.1 cm), foot (total length of hand (phalanges and metacarpals included to the tarsal joint, not including claw) +/-0.1 cm), tibia ((calcaneal tuber to the proximal tibial tuberosity) +/-0.1 cm), and femur ((greater trochanter of the femur to the distal point of the lateral condyle of the femur) +/-0.1 cm)); testes ((width and length of the right and left testes in males) +/-0.1 mm); and upper and lower canines ((length of the upper and lower right canines from the tip to the gum line) +/-0.1 mm). For presentation purposes, we present average measurements and standard deviations of the weight, head crown, body length, tail length, forelimb, and hindlimb in this publication following the guidelines of Smith and Jungers (1997; Table 1A-B). All other measurements, e-voucher photographs, and data are available at http://www.omahazoo.com/ccr/index.asp?page=/ccr/genetics/genhome.htm (Appendix Ia). All traits were tested for significance among species using Analysis of Variance (ANOVA) performed using an online statistical service (Kirkman 1996). Species means were plotted in histogram form with the appropriate 95% confidence intervals (Appendix Id).

Data Collection.—To correlate our data with previously published molecular studies, we analyzed the following regions of the mitochondrial DNA (mtDNA): the displacement loop or control region (D-loop; Baker et al. 1993; Wyner et al. 1999) and a fragment of the cytochrome oxidase subunit III gene (COIII), NADH-dehydrogenase subunits 3, 4L, and 4 (ND3, ND4L, and ND4), as well as the $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$ genes (subsequently referred to as the PAST fragment; Pastorini et al. 2000, 2003). Using 50 nanograms of genomic DNA, the D-loop (562-563 base pairs (bp)) and the PAST (2380 bp) fragments were amplified using the following conditions: 94° C for 30 sec, 47° C for 45 sec, 72° C for 45 sec for 34 cycles. Since potential nuclear insertions or mitochondrial pseudogenes within the nuclear genome can be amplified inadvertently, we chose to minimize this likelihood by amplifying both mitochondrial DNA regions as intersecting or overlapping segments (Zhang and Hewitt 1996). Consequently, the PAST fragment was generated from six amplified segments. Additionally, to further eliminate amplification of nuclear insertions, a technique that is species independent and both rapid and efficient derived from the degenerate oligonucleotide-primed PCR method (DOP-PCR;

Telenius et al. 1992) was used to generate the PCR products. Adapting this LL-DOP-PCR (long products from low quantity), the sequence data were generated for the D-loop fragments and PAST sequence generated from overlapping segments were confirmed. The samples were electrophoresed on a 1.2% agarose gel to verify the PCR product and purified using QIAquick PCR purification kit (Qiagen; Valencia, California). Using the BigDye terminator cycle sequencing ready reaction kit by Applied Biosystems the sequence was analyzed by capillary electrophoresis with an Applied Biosystems Prizm 3100 genetic analyzer (Applied Biosystems, Inc.; Foster City, California). A suite of internal sequencing primers from Pastorini et al. (2000, 2001) were used to generate the PAST fragment. Additionally, PCR and sequencing primers specific for genus *Avahi* were designed for the D-Loop fragment, and PCR and sequencing primers specific for ND4 region were utilized for the PAST fragments (Arevalo et al. 1994; Appendix Ib). The sequence fragments were aligned to generate a consensus sequence using Sequencher (Gene Corp; Ann Arbor, Michigan), and the consensus sequences were aligned using ClustalX (Thompson et al. 1997). All aligned sequences are available from the first author upon request. All sequences have been deposited in GenBank and the sequence data and information are available from the referenced accession numbers (Appendix II).

Phylogenetic Analysis.—Maximum-parsimony analyses (MP) were performed for the phylogenetic study of the D-loop, PAST, and combined (D-Loop and PAST fragments) sequence data with PAUP Version 4.0b10 software (Swofford 2001). Heuristic searches were completed using the random addition sequence (1000 replicates) with the tree bisection-reconnection branch swapping routine. Gaps were considered as a fifth character in MP analyses, but were treated as missing data in the NJ analyses. Bootstrap analyses were accomplished with 1000, 3000, and 4000 pseudoreplicates with the D-loop, PAST, and combined data sets, respectively. Only nodes with greater than 50% support were reported. The maximum likelihood (ML) analyses were performed using the program PAUP Version 4.0b10 software (Swofford 2001). Due to the large number of taxa and characters and the resulting computational intensity, we pruned the combined sequence dataset by choosing taxa representing distinct haplotypes supported in an initial neighbor-joining (NJ; Saitou and Nei 1987) analysis.

Bayesians inference analyses were conducted using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The model of evolution was selected by using MrModeltest 2.2, a modified version of Modeltest 3.6 (Posada and Crandall 1998; Nylander 2004). A Markov Chain Monte Carlo (MCMC) run with four simultaneous chains and 1,000,000 generations was performed. Every hundredth generation, the tree with the best likelihood score was saved, resulting in 4000 trees. The 4000 trees were condensed in a majority rule consensus tree using PAUP Version 4.0b10 software (Swofford 2001). Branch supports were assigned as posterior probabilities on the consensus tree. The pattern of sequence evolution was estimated by conducting a minimum spanning network generated with the program NETWORK Version 4.11 (Gonzales et al. 1998; Bandelt et al. 1999; Forster et al. 2001) and Arlequin, Version 2.0 (Schneider et al. 2000).

In addition to character-based phylogenetic analysis of DNA sequences, PAUP software (Swofford 2001) was also used to calculate uncorrected pairwise distances for D-Loop, and PAST combined fragments ('p'). As described in Davis and Nixon (1992), Wyner et al. (1999), Mayor et al. (2004), Andriantompohavana et al. (2006), and Louis et al. (2006a, 2006b) we utilized MacClade 3.01 (Maddison and Maddison 1992) and MEGA Version 2.0 (Kumar et al. 1993) in a diagnostic search to designate evolutionary significant units (ESU) using population aggregate analysis (PAA) of the D-loop (562-563 bp) and PAST (2380 bp) sequence data for *Avahi*. With the sequential addition of each individual without an *a priori* species designation, a PAA distinguishes attributes or apomorphic characters according to the smallest definable unit (Davis and Nixon 1992; Mayor et al. 2004; Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b).

RESULTS

Following ANOVA among the species, subspecies and two additional types, only the tail length was found to be nonsignificant. The body length was slightly significant ($P<0.05$), the head crown, humerus and femur lengths were moderately significant ($P<0.01$), while all other measurements were strongly to highly significant ($P<0.001$). Means and standard deviations are reported in Tables 1A and 1B; histograms defining the 95% confidence intervals for all measurements are located in Appendix Id.

Mitochondrial DNA sequence data were completed for two fragments, D-loop and PAST fragment (approximately 2380 bp) for 98 individuals, representing all six recognized species of woolly lemurs from a total of twelve sites (Fig. 1; Appendix II). All new mtDNA sequences generated for this study were deposited in GenBank and can be acquired through the accession number (Appendix Ia; Appendix II). The sequence alignments for the data sets are available from the first author upon request. The PAST fragment consists of the 3' end of the COIII gene (30 bp), the complete NADH-dehydrogenase subunits ND3 (348 bp), ND4L (297 bp), and ND4 (1378 bp), along with the tRNA genes, glycine (73 bp), arginine (73 bp), histidine (70 bp), serine (65 bp), and the 5' portion of leucine (47 bp). The polyadenylation of COIII and ND4 genes, insertion of base pairs between ingroup/outgroup comparisons, and other alignment characteristics between lemurs and *Homo* are consistent with Pastorini et al. (2000).

Based on the phylogenetic inferences of the NJ, MP, and ML analyses of three sequence alignments (D-loop, PAST, and D-Loop-PAST fragment combined), two major *Avahi* subgroups are represented, differentiating the six recognized woolly lemur species (Figs. 8-14). The first subgroup corresponds to the western woolly lemurs, *A. occidentalis*, *A. cleesei*, and *A. unicolor* (Fig. 8). The second subgroup includes the eastern woolly lemurs, *A. meridionalis meridionalis*, *A. m. ramanantsoavana*, *A. peyrierasi*, and *A. laniger* (Fig. 8). Two haplotype groups or types of *A. peyrierasi* were represented within the eastern woolly lemurs (Figs. 8-14). In addition, another group of woolly lemurs from the Fandriana region formed a distinct clade, *A.* species *nova* #1, from the other recognized species (Fig. 8). There is high bootstrap support for the MP and NJ analysis with respect to the topology of the genera and species as every major node between the species clusters has a greater than 50% bootstrap support (Figs. 8, 11, and 13). The results from the population aggregate analysis of the D-loop and PAST sequence data are presented in Tables 2A-B and 3A-B, respectively. Multiple diagnostic characters define each *Avahi* species, along with two types of *Avahi laniger* (only the results from the PAST fragment PAA designates attributes specific for each region), two types of *Avahi peyrierasi*, and the one newly described species, *A.* species *nova* #1 (Figs. 15-17; Tables 2 and 3). A review of the morphometric data for the six recognized species of woolly lemurs are summarized in Table 1 and are presented in Appendix Id, along with the two types of *A. peyrierasi*, and the newly described species (Appendix Ia; detailed morphological measurements of the individual animals are available at http://www.omahazoo.com/ccr/index.asp?page=/ccr/genetics/genhome.htm). The uncorrected 'p' distance and the absolute genetic differences are presented in Table 4A-B. The highest average uncorrected pairwise distances and absolute genetic differences for the D-Loop sequence alignment were between the western and eastern woolly lemurs, ranging from 12.28 to 8.84 and 65 to 47 (*A. cleesei* to *A. peyrierasi* type #2 and *A. occidentalis* to *A. peyrierasi* #3), respectively. The highest average uncorrected pairwise distances and absolute genetic differences for the PAST sequence alignment were between the western and eastern woolly lemurs, ranging from 11.72 to 10.20 and 251 to 220 (*A. occidentalis* to *A. m. meridionalis* and *A. occidentalis* to *A. peyrierasi* #3), respectively. The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the D-Loop sequence alignment between the eastern woolly lemurs range from 5.99 to 3.43 and 43 to 19 (*A. m. meridionalis* to *A. laniger* and *A.* species

(text continued on page 35)

*Table 1A. Morphometric data collected from sedated individuals. (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1. Individual morphological data available online; see Appendix I).*

Species Name	Common Name	N	Weight (kg)	Head Crown (cm)	Body Length (cm)	Tail Length (cm)
Avahi cleesei	Cleese's woolly lemur	5	0.86±0.11	7.3±1.2	24.4±4.0	32.2±2.4
Avahi occidentalis	western woolly lemur	8	0.94±0.10	6.4±0.5	22.4±1.2	34.2±3.3
Avahi unicolor	Sambirano woolly lemur	6	0.84±0.12	6.5±0.2	22.0±1.6	33.3±2.1
Avahi laniger	eastern woolly lemur	24	1.13±0.25	6.9±0.6	22.8±1.9	33.0±3.8
Avahi peyrierasi type #2	Peyrieras' woolly lemur (Type #2)	7	1.07±0.14	7.2±0.3	21.4±2.0	33.4±2.9
Avahi peyrierasi type #3	Peyrieras' woolly lemur (Type #3)	9	0.98±0.27	6.9±2.4	21.1±5.5	31.1±7.8
Avahi meridionalis ramanantsoavana	Ramanantsoavana's woolly lemur	10	0.98±0.13	7.1±1.0	23.2±2.0	32.4±6.4
Avahi meridionalis meridionalis	southern woolly lemur	10	1.06±0.17	6.5±0.1	24.2±1.5	33.4±3.7
Avahi betsileo	Betsileo woolly lemur	9	1.05±0.16	7.6±1.6	22.1±2.3	31.4±2.9

*Table 1B. Morphometric data collected from sedated individuals. (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1. Individual morphological data available online; see Appendix I).*

Species Name	Forelimb					Hindlimb				
	Thumb Length (cm)	LD Length (cm)	Hand Length (cm)	Ulna/Radius Length (cm)	Humerus Length (cm)	Hallux Length (cm)	LD Length (cm)	Foot Length (cm)	Tibia Length (cm)	Femur Length (cm)
Avahi cleesei	2.6±0.2	3.0±0.1	5.8±1.3	8.5±0.7	6.5±0.3	4.9±0.2	2.9±0.2	8.6±1.4	11.7±0.4	13.2±0.4
Avahi occidentalis	2.0±0.2	2.7±0.2	6.3±0.4	7.6±0.4	6.1±0.6	3.9±0.8	2.4±0.5	8.3±0.3	10.2±0.9	12.4±0.3
Avahi unicolor	2.3±0.2	3.0±0.4	6.4±0.3	8.3±0.4	5.9±0.6	3.9±0.5	3.1±0.1	8.7±0.3	10.3±0.3	12.6±0.6
Avahi laniger	2.6±0.4	3.5±0.5	7.2±0.7	8.6±0.8	6.5±0.8	5.1±0.4	3.2±0.5	9.8±0.6	11.4±1.0	13.2±1.3
Avahi peyrierasi type #2	1.9±0.2	3.3±0.3	7.1±0.2	8.3±0.4	6.1±0.6	3.4±0.6	3.0±0.3	9.6±0.5	10.8±0.5	13.8±0.5
Avahi peyrierasi type #3	2.0±0.7	3.2±1.1	7.0±2.4	8.2±2.3	5.6±1.8	3.4±1.3	3.0±1.2	9.5±2.6	10.4±3.1	13.8±3.7
Avahi m. ramanantsoavana	2.5±0.0	3.7±0.7	6.6±0.2	8.6±0.5	6.6±0.6	5.0±0.1	4.0±1.5	9.4±0.1	11.0±1.2	12.9±0.6
Avahi m. meridionalis	2.6±0.1	3.4±0.2	6.5±0.6	9.3±0.6	6.3±0.5	5.3±0.3	3.3±0.3	10.0±0.6	11.1±0.5	13.9±0.8
Avahi betsileo	2.6±0.3	3.3±0.2	5.5±1.9	8.6±0.7	6.2±0.8	5.1±0.3	3.2±0.3	8.6±1.6	11.4±1.4	13.4±0.9

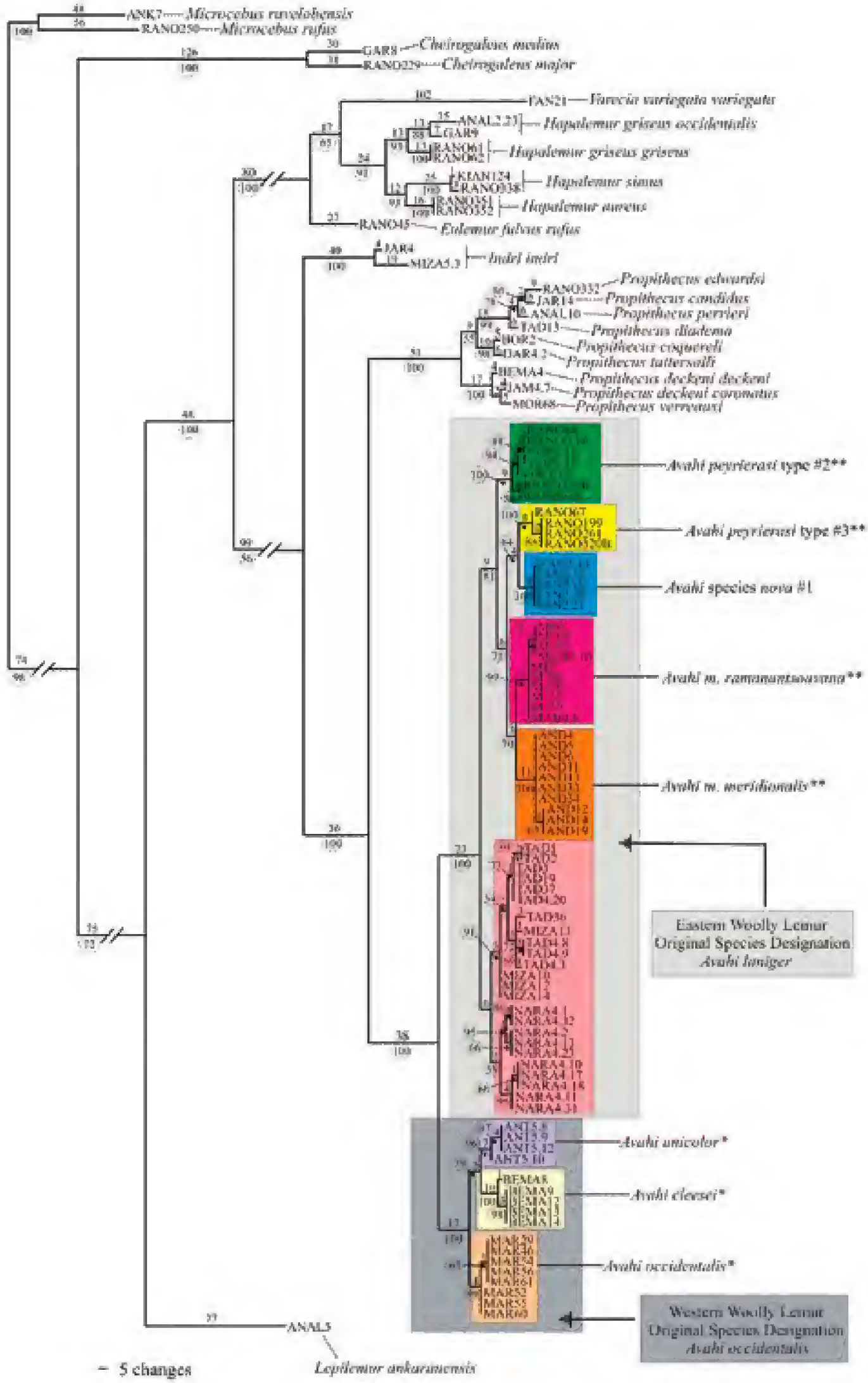

Figure 8. Neighbor-joining phylogram derived from the D-loop DNA sequence data from the 77 *Avahi* individuals with 26 out-group taxa. Species designated according to the distribution in the current literature (Mittermeier et al. 2006; *Thalmann and Geissmann 2000; *Thalmann and Geissmann 2005; **Zaramody et al. 2006). Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates. *Avahi* catalogue numbers that are listed in Appendix II, but are not shown in this figure, are presented as haplotype information in Appendix 1c.

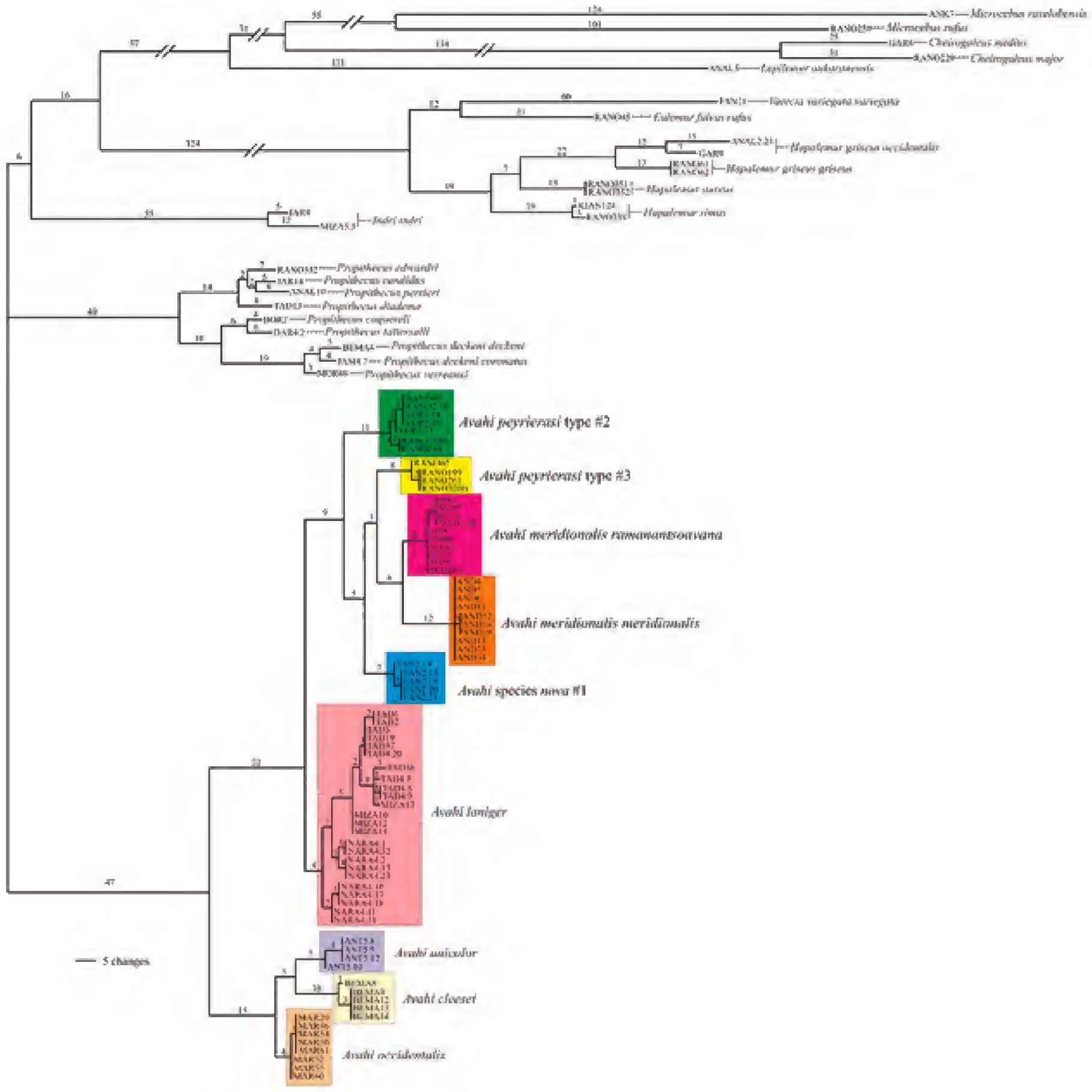

Figure 9. Maximum parsimony phylogram derived from D-loop fragment sequence data from 77 *Avahi* individuals (one of 144 most parsimonious trees). Values above branches indicate number of changes between nodes. Length = 1,271; CI = 0.5382; RI = 0.8786; RC = 0.4728; HI = 0.4618.

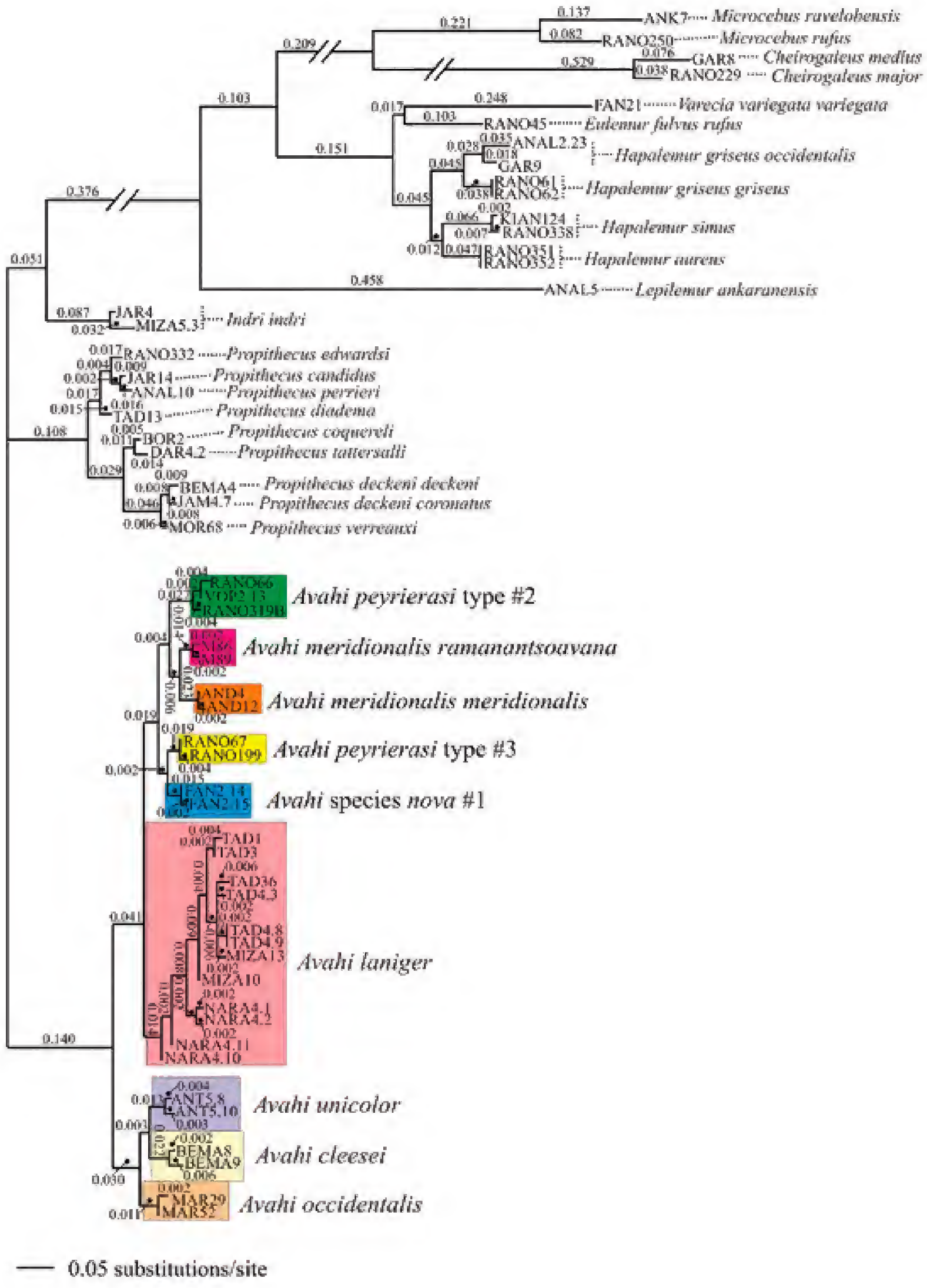

Figure 10. Maximum-likelihood phylogram derived from D-loop sequence data from 29 *Avahi* haplotypes. The phylogram presented with branch lengths proportional to the number of changes (values specified on the branches). We obtained the maximum likelihood phylogram (-1n likelihood = 6398.15) from the D-loop alignment from a transition/transversions ratio of 2.28 ($\kappa = 4.66$) and γ shape parameter of 0.54. (FAN6.6 and FAN6.7 are identical to haplotype FAN2.14; FAN6.9, FAN6.10, and FAN6.11 are identical to haplotype FAN2.15; these individuals are not displayed in any of the figures).

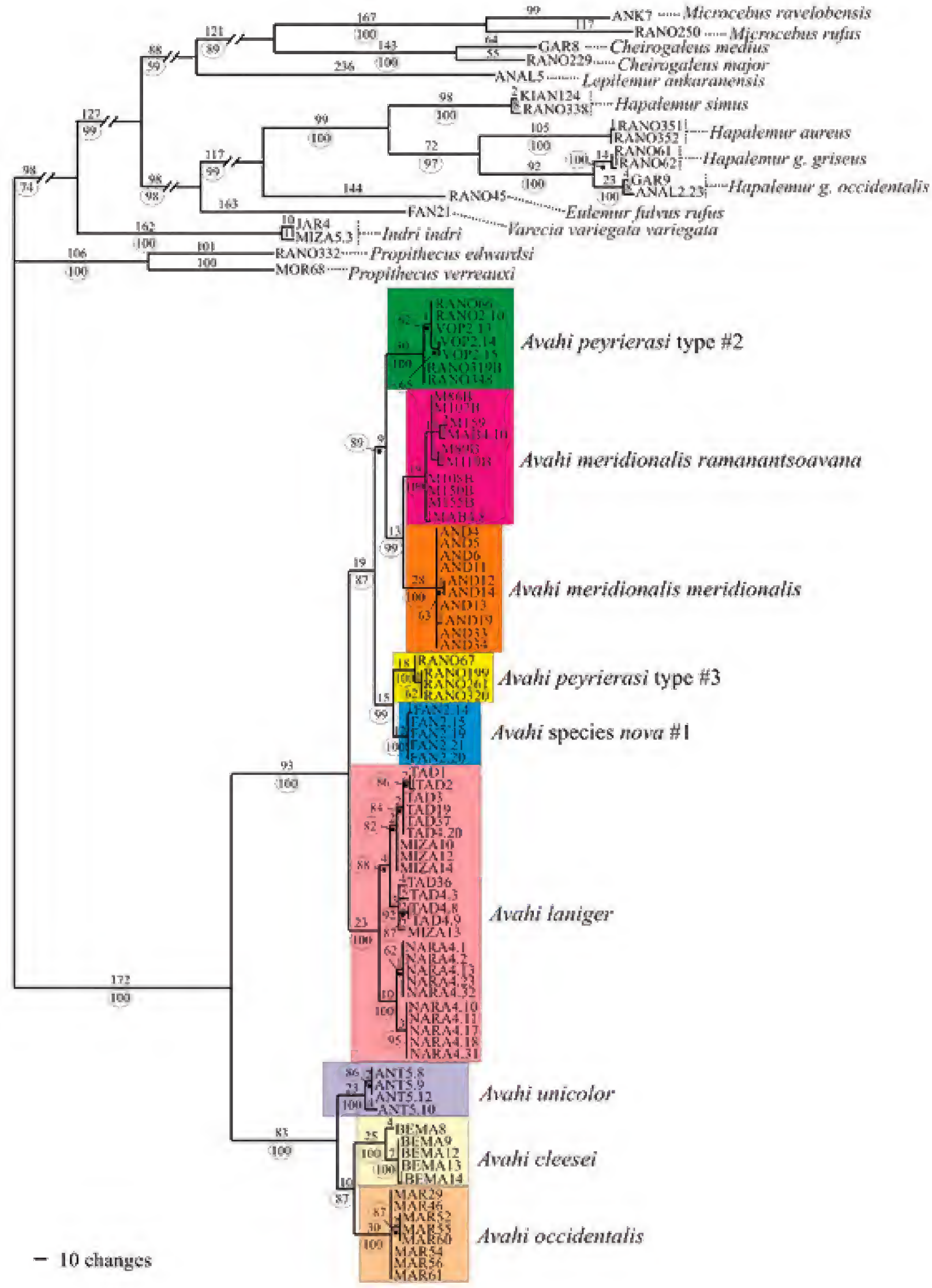

Figure 11. Maximum parsimony phylogram derived from PAST fragment sequence data from 77 *Avahi* individuals (one of 53 most parsimonious trees). Values above branches indicate number of changes between nodes. Length = 3,551; CI = 0.4816; RI = 0.8721; RC = 0.4200; HI = 0.5184.

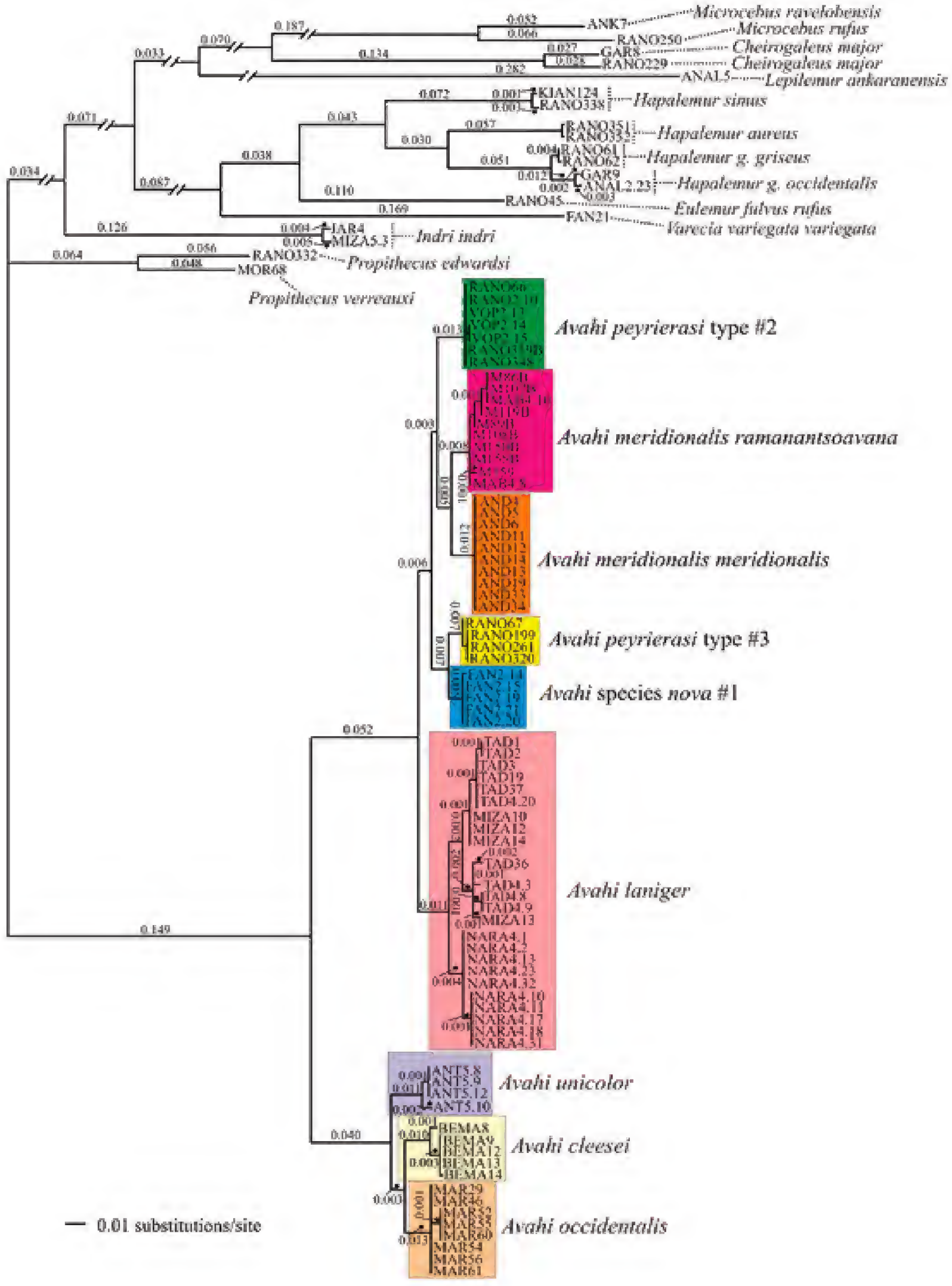

Figure 12. Maximum-likelihood phylogram derived from PAST sequence data from 77 *Avahi* individuals. The phylogram presented with branch lengths proportional to the number of changes (values specified on the branches). We obtained the maximum likelihood phylogram (-1n likelihood = 18,123.61) from the PAST alignment from a transition/transversions ratio of 5.94 ($\kappa = 12.31$) and γ shape parameter of 0.34.

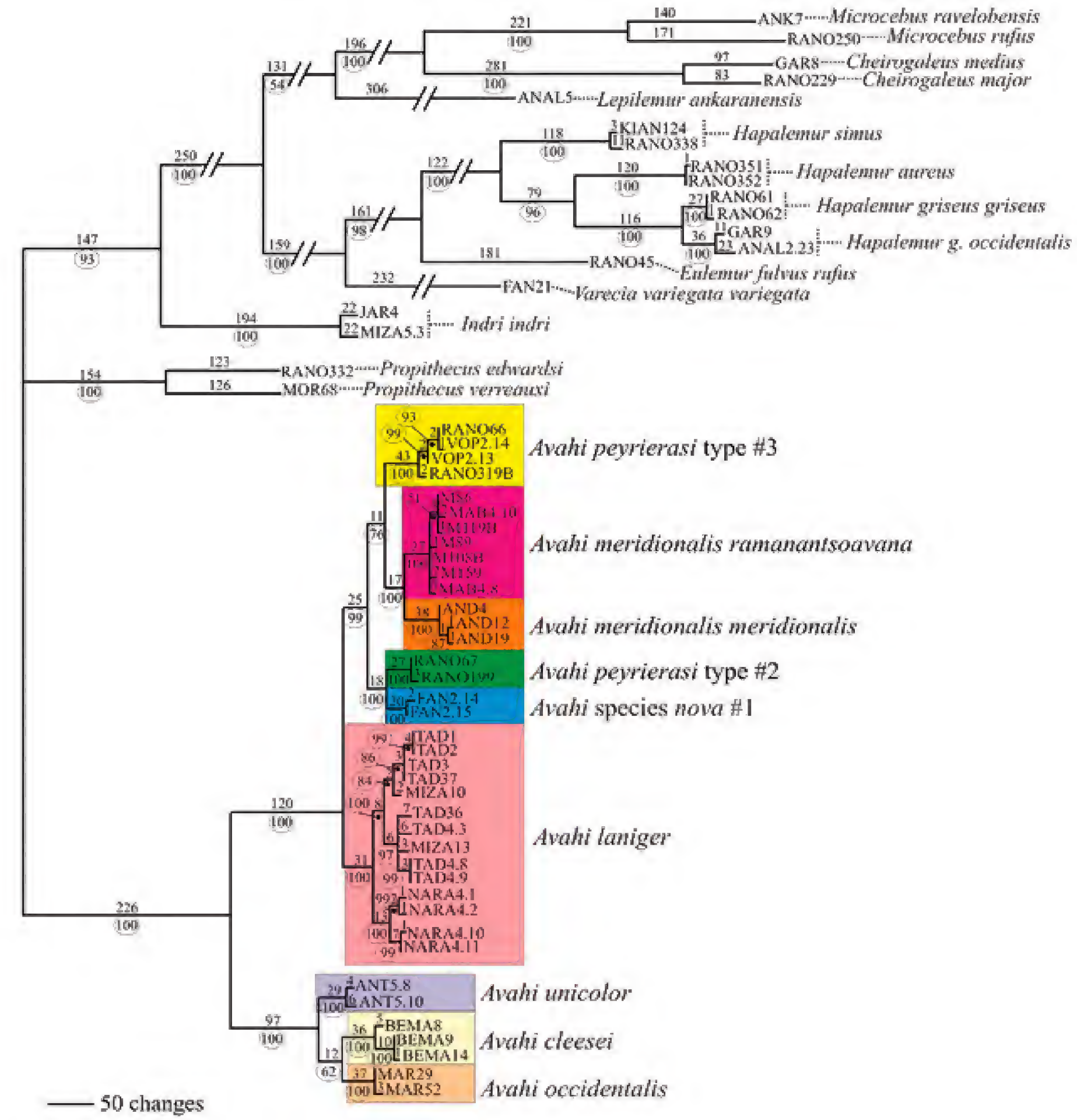

Figure 13. Neighbor-joining phylogram derived from the D-loop and PAST combined DNA sequence data from the 39 *Avahi* haplotypes with 19 out-group taxa. Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates.

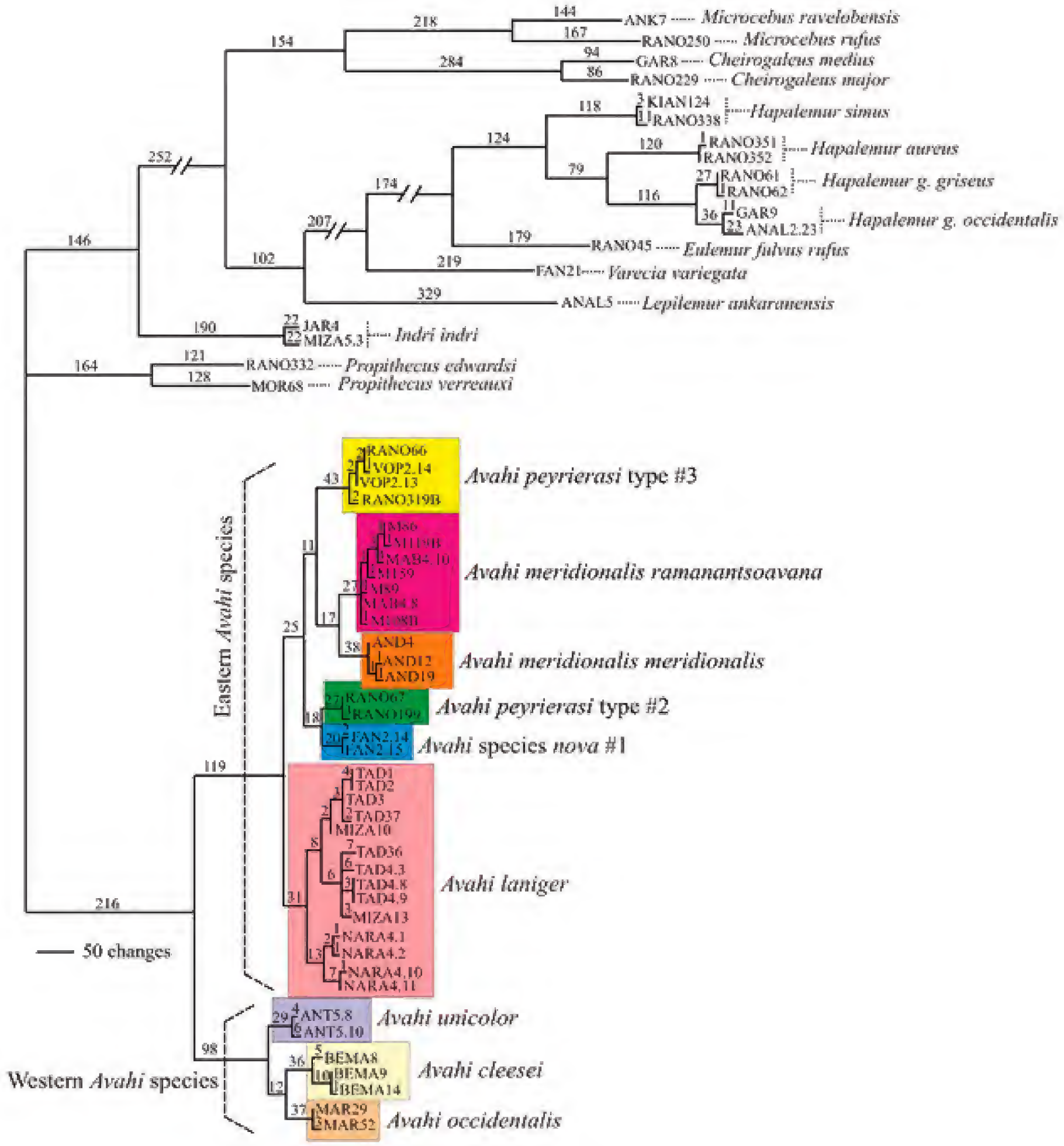

Figure 14. Maximum parsimony phylogram derived from the D-loop and PAST combined DNA sequence data from the 39 *Avahi* haplotypes with 19 out-group taxa (one of 20 most parsimonious trees). Values above branches indicate number of changes between nodes. Length = 4,999; CI = 0.5141; RI = 0.8392; RC = 0.4314; HI = 0.4859.

Table 2A. Diagnostic nucleotide sites from the D-Loop Pairwise Aggregate Analysis (PAA) of Avahi.

	1111111122222222333333333334445555555
	1260155799927778899000113333499029000022 44
	6046289245975679785602934234686712925671538
RANO66	ATCGAAAACACAGGAATTTAAGTCCTCTACAGCCAAAAATCGG
RANO319B	.....C.....
RANO348	.....C.....
RANO2.10	.....
VOP2.13	.....
VOP2.14	.....
VOP2.15	.....
RANO67	.....TTC....T.....T.G.....GG....C
RANO199	.....TTC....T.....T.G.....GG....C
RANO320B	.....TTC....T.....T.G.....GG....C
RANO261	.....TTC....T.....T.G.....GG....C
M86	.....G.TACT...T.....T.....GG...T..
M89	.....G.TACT...T.....T.....GG...T..
M107	.....G.TACT...T.....T.....GG...T..
M108	.....G.TACT...T.....T.....GG...T..
M119	.....G.TACT...T.....T.....GG...T..
M150	.....G.TACT...T.....T.....GG...T..
M155	.....G.TACT...T.....T.....GG...T..
M159	.....G.TACT...T.....T.....GG...T..
MAB4.8	.....G.TACT...T.....T.....GG...T..
MAB4.10	.....G.TACT...T.....T.....GG...T..
FAN2.14	.C.....TTC.G..T.....C...TC.T.....GG..C...
FAN2.15	.C.....TTC.G..T.....C...TC.T.....GG..C...
FAN2.19	.C.....TTC.G..T.....C...TC.T.....GG..C...
FAN2.20	.C.....TTC.G..T.....C...TC.T.....GG..C...
FAN2.21	.C.....TTC.G..T.....C...TC.T.....GG..C...
TAD1	.....TTC..C..T.....T.....GG....A.
TAD2	.....TTC..C..T.....T.....GG....A.
TAD3	.....TTC..C..T.....T.....GG....A.
TAD19	.....TTC..C..T.....T.....GG....A.
TAD36	....G..TTC..C..T.....T.....GG....A.
TAD37	.....TTC..C..T.....T.....GG....A.
TAD4.3	....G..TTC..C..T.....T.....GG....A.
TAD4.8	....G..TTC..C..T.....T.....GG....A.
TAD4.9	....G..TTC..C..T.....T.....GG....A.
TAD4.20	.....TTC..C..T.....T.....GG....A.
MIZA10	.....TTC..C..T.....T.....GG....A.
MIZA12	.....TTC..C..T.....T.....GG....A.
MIZA14	.....TTC..C..T.....T.....GG....A.
MIZA13	....G..TTC..C..T.....T.....GG....A.

Table 2A. (cont.)

	1111111122222222233333333333444555555555
	1260155799927778899000113333499029000022444
	6046289245975679785602934234686712925671538
AND4	G.T.G.GTTC.....T..........T......T.GGG.....
AND5	G.T.G.GTTC.....T..........T......T.GGG.....
AND6	G.T.G.GTTC.....T..........T......T.GGG.....
AND11	G.T.G.GTTC.....T..........T......T.GGG.....
AND12	G.T.G.GTTC.....T..........T......T.GGG.....
AND13	G.T.G.GTTC.....T..........T......T.GGG.....
AND14	G.T.G.GTTC.....T..........T......T.GGG.....
AND19	G.T.G.GTTC.....T..........T......T.GGG.....
AND33	G.T.G.GTTC.....T..........T......T.GGG.....
AND34	G.T.G.GTTC.....T..........T......T.GGG.....
NARA4.1	.......TTC..C..T..........T........GG.....
NARA4.2	.......TTC..C..T..........T........GG.....
NARA4.10	.......TTC..C..T..........T........GG.....
NARA4.11	.......TTC..C..T..........T........GG.....
NARA4.13	.......TTC..C..T..........T........GG.....
NARA4.17	.......TTC..C..T..........T........GG.....
NARA4.18	.......TTC..C..T..........T........GG.....
NARA4.23	.......TTC..C..T..........T........GG.....
NARA4.31	.......TTC..C..T..........T........GG.....
NARA4.32	.......TTC..C..T..........T........GG.....
ANT5.8	...A...TTC..C..T.C.....T..TG..GAT..GG.....
ANT5.9	...A...TTC..C..T.C.....T..TG..GAT..GG.....
ANT5.10	...A...TTC..C..T.C.....T..TG..GAT..GG.....
ANT5.12	...A...TTC..C..T.C.....T..TG..GAT..GG.....
BEMA8	..A.C..TTC..AA.CC...GA...CTG...C...GG.....
BEMA9	..A.C..TTC..AA.CC...GA...CTG...C...GG.....
BEMA12	..A.C..TTC..AA.CC...GA...CTG...C...GG.....
BEMA13	..A.C..TTC..AA.CC...GA...CTG...C...GG.....
BEMA14	..A.C..TTC..AA.CC...GA...CTG...C...GG.....
MAR29	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR46	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR52	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR54	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR55	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR56	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR60	.......TTC..C..T..CG....T.TG...C..GGG.....
MAR61	.......TTC..C..T..CG....T.TG...C..GGG.....

Table 2B. Diagnostic nucleotide sites from the PAST Pairwise Aggregate Analysis (PAA) of Avahi.

```
                  1111111122222233355555556666777777888899999999999999
                12457890011123514578933400247793679000688233411223344555678
              68497455328148135368991694012026065181484356587060858052782 25
RANO66        GCTATAAAACCGATAGCGAACCACAACTCAGCACAGCCACTTTCCGACCGTACCACATTAC
RANO319b      ..........................................................
RANO348       ..........................................................
RANO2.10      ..........................................................
VOP2.13       ..........................................................
VOP2.14       ..........................................................
VOP2.15       ..........................................................
RANO67        ..C..GC...A.....T.G......C....A.C...TT................G...C..
RANO199       ..C..GC...A.....T.G......C....A.C...TT................G...C..
RANO261       ..C..GC...A.....T.G......C....A.C...TT................G...C..
RANO320       ..C..GC...A.....T.G......C....A.C...TT................G...C..
M86b          ..CG..C.........T...T....C....A.C...T................TTG....GT
M89b          ..CG..C.........T...T....C....A.C...T................TTG....GT
M107b         ..CG..C.........T...T....C....A.C...T................TTG....GT
M108b         ..CG..C.........T...T....C....A.C...T................TTG....GT
M119b         ..CG..C.........T...T....C....A.C...T................TTG....GT
M150b         ..CG..C.........T...T....C....A.C...T................TTG....GT
M155b         ..CG..C.........T...T....C....A.C...T................TTG....GT
M159          ..CG..C.........T...T....C....A.C...T................TTG....GT
MAB4.8        ..CG..C.........T...T....C....A.C...T................TTG....GT
MAB4.10       ..CG..C.........T...T....C....A.C...T................TTG....GT
FAN2.14       ..C...C...A.....T......T.C....A.C...T........A...A....G......
FAN2.15       ..C...C...A.....T......T.C....A.C...T........A...A....G......
FAN2.19       ..C...C...A.....T......T.C....A.C...T........A...A....G......
FAN2.21       ..C...C...A.....T......T.C....A.C...T........A...A....G......
```

Table 2B. (cont.)

```
                  1111111122222233355555556666777777888899999999999999
                124578900111235145789334002477936790006882334112233445556 78
               684974553281481353689916940120260651814843565870608580527822 5
FAN2.20        ..C...C...A.....T......T.C....A.C...T........A...A....G...... .
TAD1           ..C...C.........T........T....A.C..AT..................G.G.... .
TAD2           ..C...C.........T........T....A.C..AT..................G.G.... .
TAD3           ..C...C.........T........T....A.C..AT..................G.G.... .
TAD19          ..C...C.........T........T....A.C..AT..................G.G.... .
TAD36          ..C...C.........T........T....A.C..AT..................G.G.... .
TAD37          ..C...C.........T........T....A.C..AT..................G.G.... .
TAD4.3         ..C...C.........T........T....A.C..AT..................G.G.... .
TAD4.8         ..C...C.........T........T....A.C..AT..................G.G.... .
TAD4.9         ..C...C.........T........T....A.C..AT..................G.G.... .
TAD4.20        ..C...C.........T........T....A.C..AT..................G.G.... .
MIZA10         ..C...C.........T........T....A.C..AT..................G.G.... .
MIZA12         ..C...C.........T........T....A.C..AT..................G.G.... .
MIZA13         ..C...C.........T........T....A.C..AT..................G.G.... .
MIZA14         ..C...C.........T........T....A.C..AT..................G.G.... .
AND4           ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND5           ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND6           ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND11          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND12          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND13          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND14          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND19          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND33          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
AND34          ..C...CG........T....TG.GC....A.C...T..A......G....T..GT.C... .
```

Table 2B. (cont.)

```
                  1111111122222233355555556666777777888999999999999999
                  1245789001112351457893340024779367900068823341223344555678
                  6849745532814813536899169401202606518148435658706085805278225
NARA4.1           ..C...C.........T........T....ATC...T.......T.........G......
NARA4.2           ..C...C.........T........T....ATC...T.......T.........G......
NARA4.10          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.11          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.13          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.17          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.18          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.23          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.31          ..C...C.........T........T....ATC...T.......T.........G......
NARA4.32          ..C...C.........T........T....ATC...T.......T.........G......
ANT5.8            A.C...C.GTT.G..AT.....T..C.C..A.TA..T..........TT.C...G......
ANT5.9            A.C...C.GTT.G..AT.....T..C.C..A.TA..T..........TT.C...G......
ANT5.10           A.C...C.GTT.G..AT.....T..C.C..A.TA..T..........TT.C...G......
ANT5.12           A.C...C.GTT.G..AT.....T..C.C..A.TA..T..........TT.C...G......
BEMA8             .AC...C..TT..C..TA.G..T..C..A.A.T...T.G...C....T..A...G......
BEMA9             .AC...C..TT..C..TA.G..T..C..A.A.T...T.G...C....T..A...G......
BEMA12            .AC...C..TT..C..TA.G..T..C..A.A.T...T.G...C....T..A...G......
BEMA13            .AC...C..TT..C..TA.G..T..C..A.A.T...T.G...C....T..A...G......
BEMA14            .AC...C..TT..C..TA.G..T..C..A.A.T...T.G...C....T..A...G......
MAR29             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR46             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR52             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR54             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR55             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR56             ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
```

Table 2B. (cont.)

```
                    1111111122222233355555556666777777888899999999999999
                  12457890011123514578933400247793679000688233411223344555678
                 68497455328148135368991694012026065181484356587060858052782 25
MAR60            ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
MAR61            ..C.C.C..TTA..G.T.....T..CA..GA.T.G.T...CC.T...T..C...G......
```

Table 2B. (cont.)

```
                 11111111111111111111111111111111111111111111111111122222222222222
                 00001111111122222333333333344444555555677788888888889900000011111133
                 55671123333616777333456889900557123588107801133467784613355911233535
                 37054741589831039369133140778497957245281673857910266030239616218315
RANO66           ACTAGATTATTCCTTCCTCATCGATACTTCAAACGAACGTAACACCGATTTTCGTCAAGCGACACCAC
RANO319b         ..............................................................................
RANO348          ..............................................................................
RANO2.10         ..............................................................................
VOP2.13          ..............................................................................
VOP2.14          ..............................................................................
VOP2.15          ..............................................................................
RANO67           ......C...A.........CG......C....TA.G.......TT..C..CAA....ATA.T..T..
RANO199          ......C...A.........CG......C....TA.G.......TT..C..CAA....ATA.T..T..
RANO261          ......C...A.........CG......C....TA.G.......TT..C..CAA....ATA.T..T..
RANO320          ......C...A.........CG......C....TA.G.......TT..C..CAA....ATA.T..T..
```

Table 2B. (cont.)

```
                1111111111111111111111111111111111111111111111111111111222222222222222
                0000111111112222233333333334444455555567778888888888990000011111133
                5567112333361677733345688990055712358810780113346778461335591123353 5
                3705474158983103936913314077849795724528167385791026603023961621831 5
M86b            ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M89b            ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M107b           ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M108b           ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M119b           ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M150b           ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M155b           ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
M159            ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
MAB4.8          ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
MAB4.10         ......C...A..C.T....CG.TC..C.....TA.......T.TT.....C.A....A.A.T.....
FAN2.14         G.....C...A.........CGT..........TA........GTTT....C.A....A.A.T.....
FAN2.15         G.....C...A.........CGT..........TA........GTTT....C.A....A.A.T.....
FAN2.19         G.....C...A.........CGT..........TA........GTTT....C.A....A.A.T.....
FAN2.21         G.....C...A.........CGT..........TA........GTTT....C.A....A.A.T.....
FAN2.20         G.....C...A.........CGT..........TA........GTTT....C.A....A.A.T.....
TAD1            .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
TAD2            .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
TAD3            .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
TAD19           .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
TAD36           .....GC...A.........CG...........TA....C....TT.....C.A....A.A.T.....
TAD37           .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
TAD4.3          .....GC...A.........CGA..........TA....C....TT.....C.A....A.A.T.....
TAD4.8          .....GC...A.........CG...........TA....C....TT.....C.A....A.A.T.....
TAD4.9          .....GC...A.........CG...........TA....C....TT.....C.A....A.A.T.....
TAD4.20         .....GC...A....T....CG...........TA....C....TT.....C.A....A.A.T.....
```

Table 2B. (cont.)

	1111111111111111111111111111111111111111111111111111122222222222222
	0000111111112222233333333334444455555567778888888888990000011111133
	5567112333361677733345688990055712358810780113346778461335591123353 5
	3705474158983103936913314077849795724528167385791026603023961621831 5
MIZA10	.....GC...A.........CG..........TA....C....TT.....C.A....A.A.T.....
MIZA12	.....GC...A.........CG..........TA....C....TT.....C.A....A.A.T.....
MIZA13	.....GC...A.........CG..........TA....C....TT.....C.A....A.A.T.....
MIZA14	.....GC...A.........CG..........TA....C....TT.....C.A....A.A.T.....
AND4	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND5	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND6	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND11	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND12	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND13	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND14	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND19	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND33	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
AND34	..C.T.CC..A...C...TGCG........T..TA...T.G...TT.....C.AC...A.A.T....T
NARA4.1	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.2	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.10	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.11	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.13	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.17	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.18	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.23	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.31	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
NARA4.32	......C...A.........CG...GT......TA....C....TT...A.C.A....A.A.T.....
ANT5.8	......C.G.A.....T...CGA...A..T...GA...A.....TTAG..CC.A.TG.A.ATTT....

Table 2B. (cont.)

	111111111111111111111111111111111111111111111111111111111222222222222222
	000011111111222223333333333444445555556777888888888899000000111111333
	556711233336167773334568899005571235881078011334677846133559112335335
	370547415898310393691331407784979572452816738579102660302396162183l5
ANT5.9	......C.G.A.....T...CGA...A..T...GA...A.....TTAG..CC.A.TG.A.ATTT....
ANT5.10	......C.G.A.....T...CGA...A..T...GA...A.....TTAG..CC.A.TG.A.ATTT....
ANT5.12	......C.G.A.....T...CGA...A..T...GA...A.....TTAG..CC.A.TG.A.ATTT....
BEMA8	.T....C...A.T..A....CGA...A......GAT.TA..G..TTA....C.A.T..A.A.T.....
BEMA9	.T....C...A.T..A....CGA...A......GAT.TA..G..TTA....C.A.T..A.A.T.....
BEMA12	.T....C...A.T..A....CGA...A......GAT.TA..G..TTA....C.A.T..A.A.T.....
BEMA13	.T....C...A.T..A....CGA...A......GAT.TA..G..TTA....C.A.T..A.A.T.....
BEMA14	.T....C...A.T..A....CGA...A......GAT.TA..G..TTA....C.A.T..A.A.T.....
MAR29	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR46	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR52	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR54	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR55	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR56	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR60	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.
MAR61	...C..C..CAT.....C..CGA...A....TGAA...A.....TTA....C.A.T.GT.AGT.T.G.

Table 3A. Summary of Population Aggregate Analysis (PAA) D-Loop diagnostic sites for Avahi *species. Refer to Table2A. *No character or attribute is available for this fragment (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1).*

Species	Fragment Size (bp)	PAA base pair location
Avahi laniger type #1 (Mantadia)	562	543
Avahi laniger type #2 (Mananara-Nord)	562	*
Avahi peyrierasi type #2	562	336, 548
Avahi peyrierasi type #3	562	172, 194, 195, 333, 502, 505
Avahi meridionalis ramanantsoavana	562	158, 194, 199, 525
Avahi meridionalis meridionalis	562	16, 64, 159, 422, 506
Avahi betsileo	562	20, 227, 277, 309, 334, 348, 521
Avahi unicolor	563	106, 288, 313, 396, 397, 401
Avahi cleesei	563	64, 112, 275, 276, 279, 287, 300, 302, 332
Avahi occidentalis	563	295, 296, 314, 499

Table 3B. Summary of Population Aggregate Analysis (PAA) PAST diagnostic sites for Avahi *species. Refer to Table2B.*

Species	Fragment Size (bp)	PAA base pair location
Avahi laniger type #1 (Mantadia)	2364	671, 957, 1117, 1708
Avahi laniger type #2 (Mananara-Nord)	2364	576, 835, 1390, 1397, 1708, 1870
Avahi peyrierasi type #2	2364	54, 108, 246, 701, 962, 1408, 1584, 1861, 1946, 2096, 2153, 1052
Avahi peyrierasi type #3	2364	14, 75, 155, 344, 572, 590, 698, 945, 1124, 1139, 1341, 1353, 1525, 1537, 1818, 1835, 1886, 1960, 2059, 2111, 2123
Avahi meridionalis ramanantsoavana	2364	29, 279, 938, 940, 972, 985, 1261, 1273, 1381, 1384, 1407, 1807
Avahi meridionalis meridionalis	2364	85, 289, 291, 339, 708, 847, 935, 952, 958, 1060, 1114, 1131, 1270, 1336, 1339, 1612, 1771, 2013, 2355
Avahi betsileo	2364	108, 336, 838, 920, 1052, 1053, 1363, 1813, 1837
Avahi unicolor	2364	6, 93, 114, 133, 501, 636, 916, 1052, 1135, 1279, 1454, 1849, 1872, 2032, 2116, 2131
Avahi cleesei	2364	8, 118, 213, 258, 522, 704, 785, 928, 1057, 1213, 1273, 1552, 1585, 1786
Avahi occidentalis	2364	47, 111, 121, 500, 540, 665, 764, 783, 826, 1075, 1138, 1168, 1333, 1477, 1519, 1525, 2053, 2059, 2116, 2138, 2331

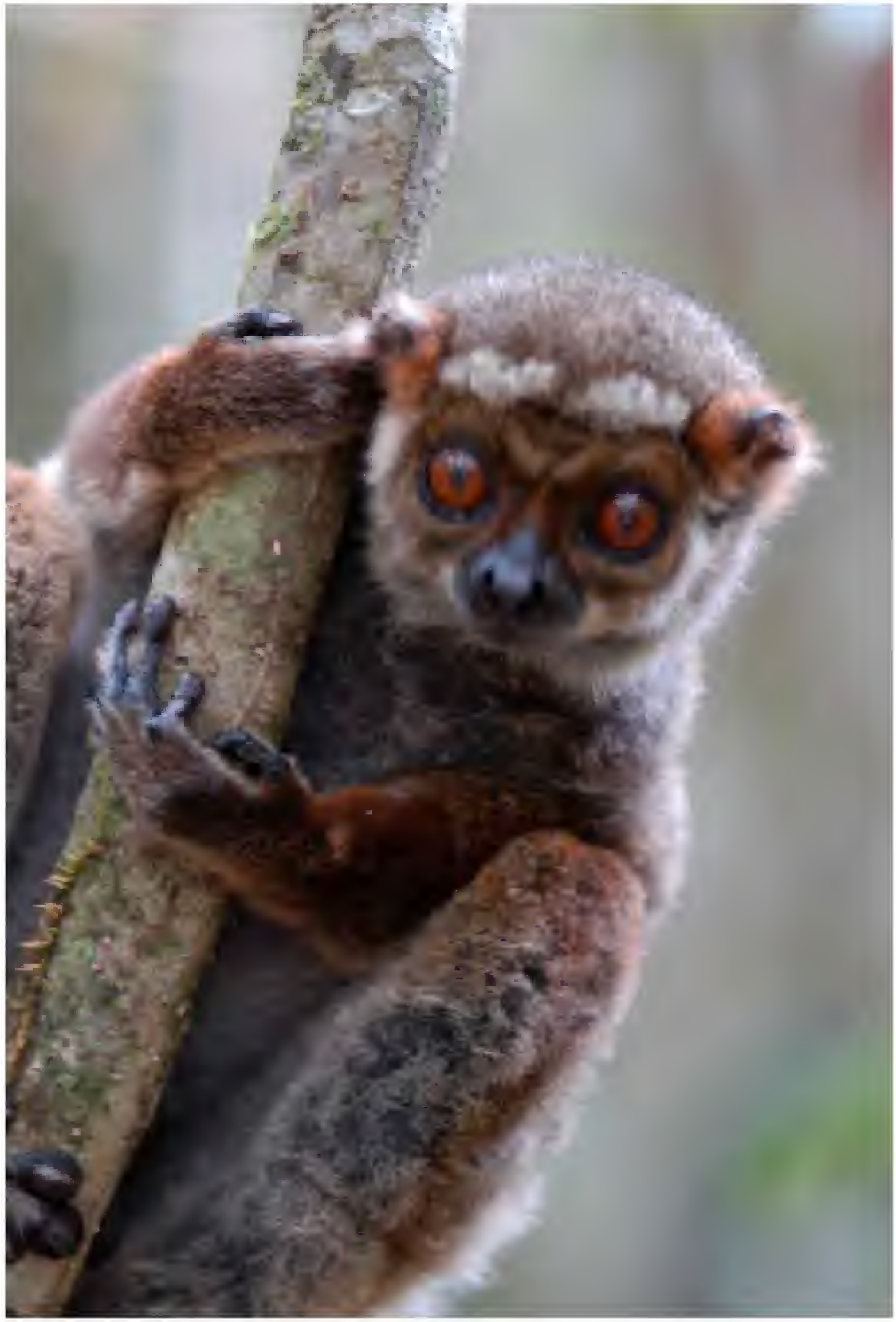

Figure 15. *Avahi laniger*, eastern woolly lemur, at Mantadia National Park. Photo by Edward E. Louis, Jr.

Figure 16. *Avahi peyrierasi* Type #2, Peyrieras' woolly lemur (Type #2), at Ranomafana National Park (Ambatolahy Dimy). Photo by Edward E. Louis, Jr.

Figure 17. *Avahi peyrierasi* Type #3, Peyrieras' woolly lemur (Type #3), at Ranomafana National Park (Ambatolahy Dimy). Photo by Edward E. Louis, Jr.

Table 4A. Genetic distance matrix for D-Loop sequence data for Avahi *species. The numbers represent the following* Avahi *species: [1]* Avahi peyrierasi *type #2; [2]* Avahi peyrierasi *type #3; [3]* Avahi ramanantsoavana; *[4]* Avahi betsileo; *[5]* Avahi meridionalis; *[6]* Avahi laniger; *[7]* Avahi unicolor; *[8]* Avahi cleesei; *and [9]* Avahi occidentalis. *Genetic distance based on absolute differences is displayed above the diagonal, and genetic distance based as a percentage is displayed below the diagonal (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1).*

	1	2	3	4	5	6	7	8	9
1		31	29	28	33	42	62	65	59
2	5.28±0.95		21	19	29	43	50	56	47
3	4.75±0.87	3.67±0.80		24	21	42	56	59	51
4	4.75±0.91	3.43±0.77	4.21±0.85		28	42	58	62	53
5	5.56±0.98	5.18±0.97	3.51±0.75	5.04±0.96		43	56	58	51
6	5.31±0.86	5.64±0.93	5.85±0.92	5.50±0.91	5.99±0.97		58	63	56
7	11.63±1.43	9.47±1.26	10.67±1.30	11.30±1.43	10.83±1.42	9.61±1.23		24	15
8	12.28±1.46	10.52±1.39	11.22±1.42	12.07±1.52	11.13±1.43	10.78±1.36	4.17±0.85		22
9	11.03±1.36	8.84±1.22	9.62±1.28	10.24±1.35	9.75±1.34	9.04±1.15	2.76±0.70	3.86±0.80	

Table 4B. Genetic distance matrix for PAST sequence data for Avahi *species. The numbers represent the following* Avahi *species: [1]* Avahi peyrierasi *type #2; [2]* Avahi peyrierasi *type #3; [3]* Avahi ramanantsoavana; *[4]* Avahi betsileo; *[5]* Avahi meridionalis; *[6]* Avahi laniger; *[7]* Avahi unicolor; *[8]* Avahi cleesei; *and [9]* Avahi occidentalis. *Genetic distance based on absolute differences is displayed above the diagonal, and genetic distance based as a percentage is displayed below the diagonal (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1).*

	1	2	3	4	5	6	7	8	9
1		75	68	68	74	105	238	244	247
2	3.21±0.36		77	31	83	103	225	236	242
3	2.78±0.36	3.24±0.41		69	52	107	239	241	249
4	2.92±0.37	1.33±0.23	2.88±0.39		74	99	220	229	235
5	3.14±0.37	3.58±0.41	2.09±0.32	3.20±0.41		110	241	246	251
6	3.78±0.37	3.66±0.35	3.75±0.36	3.45±0.37	3.99±0.37		246	247	251
7	11.06±0.68	10.46±0.68	11.06±0.69	10.20±0.66	11.29±0.71	10.69±0.65		67	66
8	11.28±0.72	10.96±0.71	11.08±0.66	10.59±0.68	11.47±0.72	10.69±0.69	2.86±0.37		64
9	11.44±0.69	11.25±0.69	11.48±0.63	10.88±0.67	11.72±0.68	10.88±0.65	2.83±0.32	2.69±0.33	

nova #1 to *A. peyrierasi* #3), respectively. The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the PAST sequence alignment between the eastern woolly lemurs range from 3.99 to 1.33 and 110 to 31 (*A. m. meridionalis* to *A. laniger* and *A.* species *nova* #1 to *A. peyrierasi* #3), respectively. The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the D-Loop sequence alignment between the western woolly lemurs range from 4.17 to 2.76 and 24 to 15 (*A. unicolor* to *A. cleesei* and *A. unicolor* to *A. occidentalis*), respectively. The highest and lowest average uncorrected pairwise distances and absolute genetic differences for the PAST sequence alignment between the western woolly lemurs range from 2.86 to 2.69 and 67 to 64 (*A. unicolor* to *A. cleesei* and *A. unicolor* to *A. occidentalis*), respectively.

The Bayesian analyses are presented in Figs. 18-19. All analyses differentiate six recognized *Avahi* species, two types of *A. peyrierasi* and the newly described species. The minimum spanning network presents diagrammatically the speciation among the six recognized woolly lemurs (Fig. 22). There is high bootstrap support for the ML analysis with respect to the topology among the genera and species (Figs. 18-19). The phylogenetic trees reconstructed through the various inferences and models produced identical topologies with the exception of the sister relationships between *A. cleesei* and *A. occidentalis* or *A. unicolor*. Based on the sequence fragment utilized to reconstruct the tree; the D-Loop sequence fragment aligned *A. cleesei* with *A. unicolor*, but the PAST sequence fragment aligned *A. cleesei* with *A. occidentalis* (D-Loop versus PAST fragments; Figs. 9-12).

DISCUSSION

Notwithstanding what previously was considered superficially inconspicuous phenotypic characters, the woolly lemurs present subtle but definable morphological and phenotypic traits (Figs. 2-7, 15-17, 21-24; Table 1; Appendix Id). Milne-Edwards and Grandidier (1875a, b) noticed *Avahi* in the northern Ampasindava area in the late the 19th century and remarked that this *Avahi* from northwest was different and smaller than other *A. laniger* but did not recognize the significance of the differentiation at that time. The trend shown by the grouping of some morphological traits and the tendency towards lighter is evident in the data analyses (Tables 1A and 1B). The tendency towards greater variation is expressed in the confidence intervals and the range of trait means observed more often among the eastern woolly lemur rather than in the western woolly lemurs demonstrated in Appendix Id. This should not be so surprising given the magnitude of variation in the mitochondrial haplotypes detected in the eastern as compared to the western species. Furthermore, the molecular sequence data submitted for the woolly lemurs corroborates the "global" phylogeographic distribution of eastern and western lemur clades (Figs. 8-14; Louis et al. 2006a, 2006b; Zaramody et al. 2006).

The D-Loop sequence presented was generated for only a single representative for each recognized species of the family Indriidae, specifically the genus *Propithecus* (data were generated for two individual samples of *Indri indri*; Figs. 8-10). The molecular sequence data offers a tentative glance at the relationships of this group to the other genera and species of lemurs. The relatively low bootstrap support (56%) between *Indri indri* and the genera *Avahi* and *Propithecus* should be further investigated, utilizing sequence data representing not only a coding region (for instance, PAST fragment or cytochrome B subunit gene), but also nuclear DNA sequence data and the addition of multiple samples from multiple subpopulations and/or localities that are representative of the recognized species' distributions (Fig. 8).

(text continued on page 43)

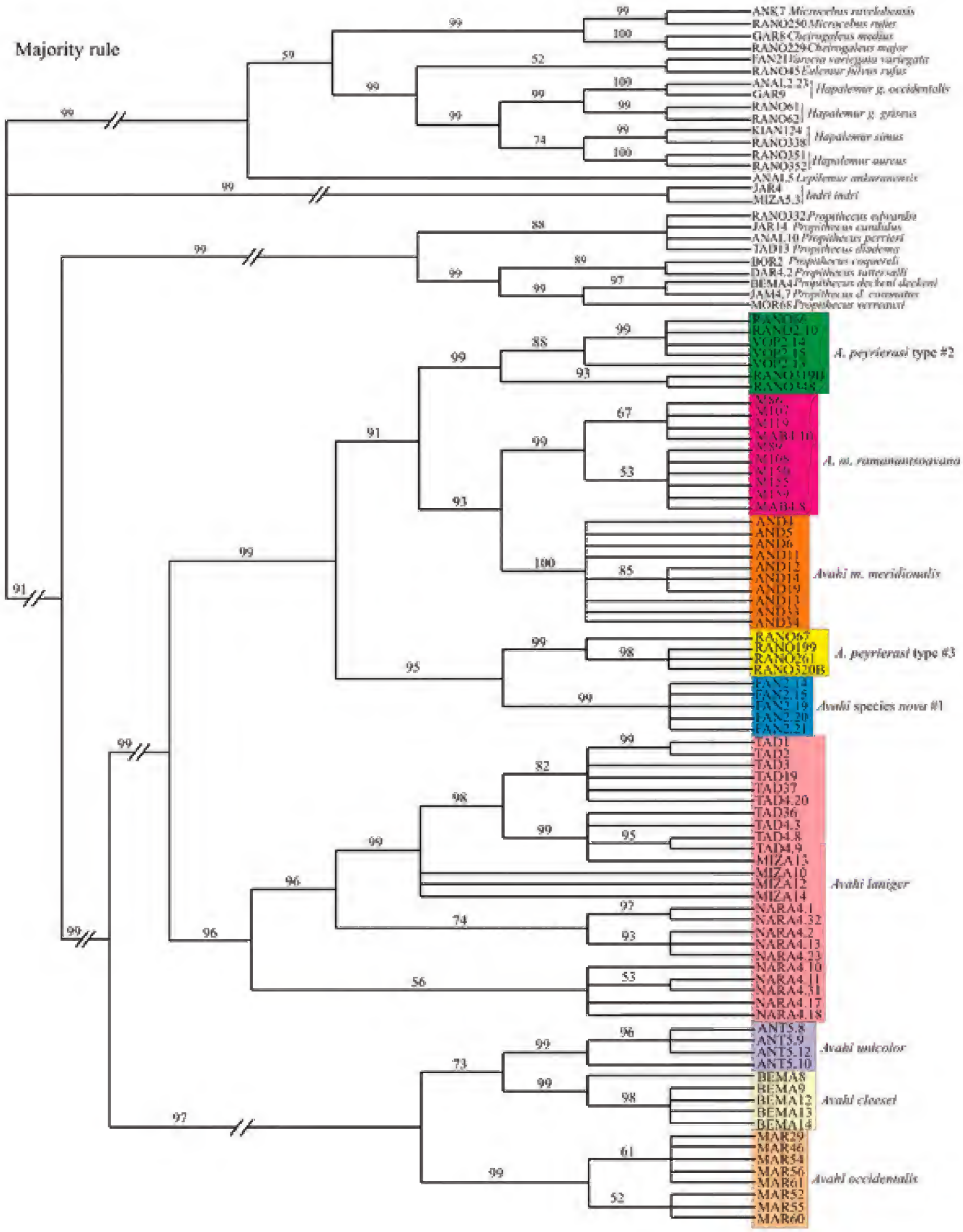

Figure 18. Bayesian majority rule maximum likelihood analysis cladogram derived from the D-loop DNA sequence data from the 77 *Avahi* individuals with 26 out-group taxa. Values above branches indicate support of bootstrap pseudoreplicates with values greater than 50% reported.

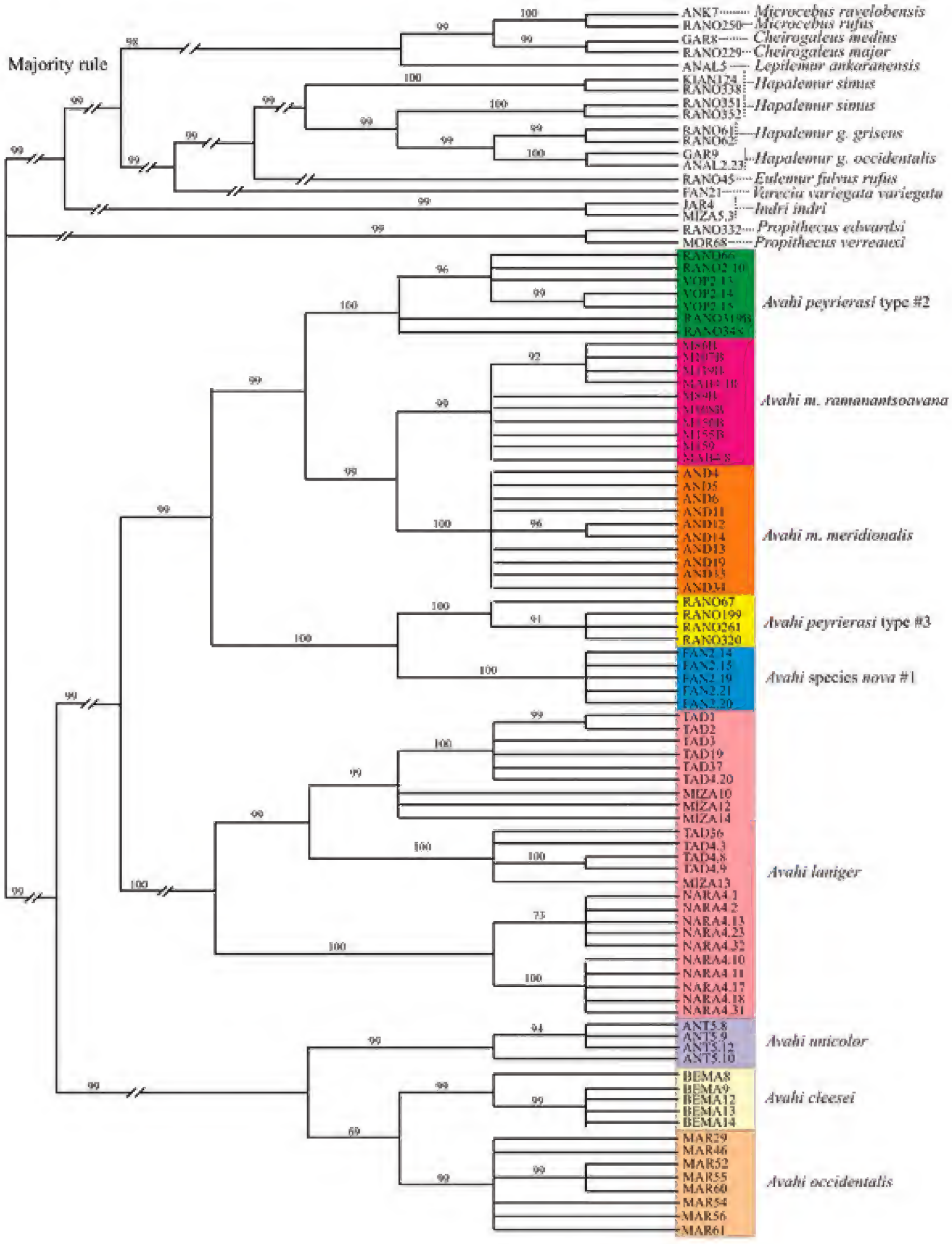

Figure 19. Bayesian majority rule maximum likelihood analysis cladogram derived from the PAST DNA sequence data from the 77 *Avahi* individuals with 19 out-group taxa. Values above branches indicate support of bootstrap pseudoreplicates with values greater than 50% reported.

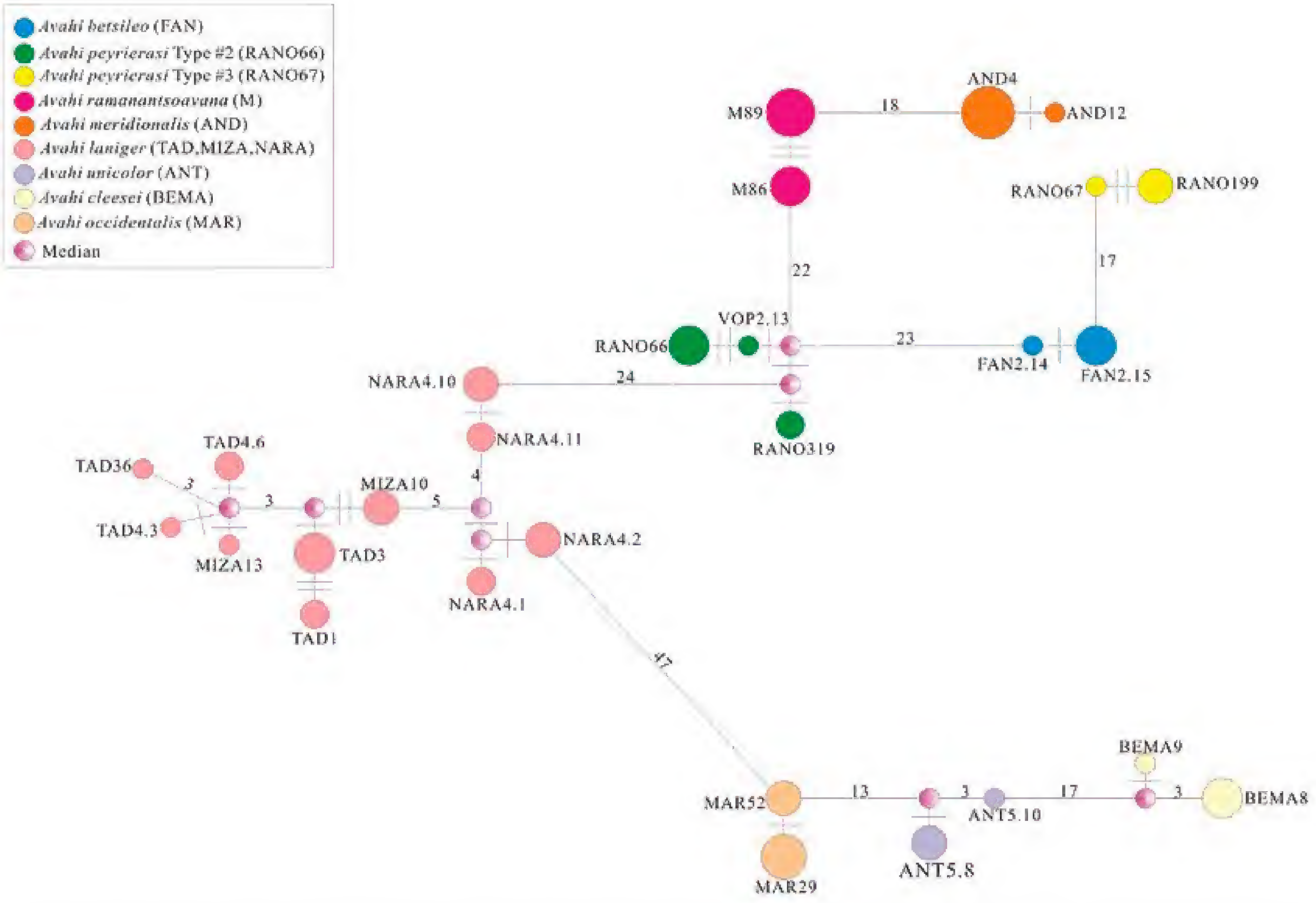

Figure 20. Minimum spanning network of Avahi haplotypes calculated using Arlequin Version 2 and Network Version 4.11. Identification numbers denote haplotypes corresponding to Appendix II. The minimum number of mutational steps separating matriarchal lines is indicated. Nucleotide substitutions are indicated by dashes. The number of nucleotide differences in their connecting lines (more than 10) is indicated when they are more than one. Missing intermediates are indicated by gray circles. The size of the circles approximates the number of individuals with matching haplotypes (circles without any number represent one individual).

Figure 21. *Avahi meridionalis*, southern woolly lemur, at Andohahela National Park (Manangotry). Photo by Edward E. Louis, Jr.

Figure 22. *Avahi ramanantsoavana,* Ramanantsoavana's woolly lemur, at Manombo Special Reserve. Photo by Richard Randriamampionona.

Figure 23. *Avahi betsilio,* Betsilio woolly lemur, at Fandriana District (Bemosary Classified Forest). *A. betsilio* was formerly referred to as *Avahi* species *nova* #1. Photo by John R. Zaonarivelo.

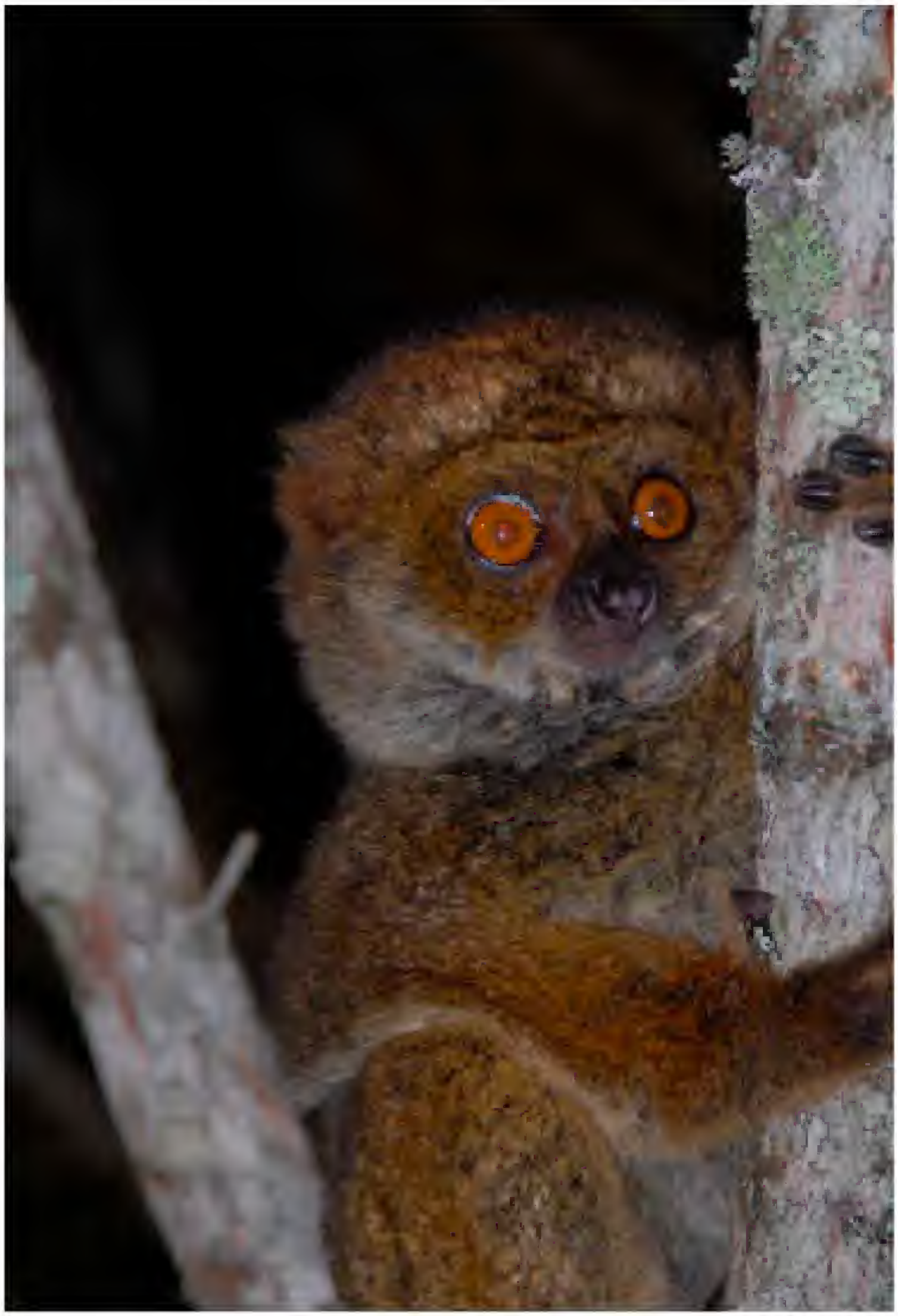

Figure 24. *Avahi betsilio,* Betsilio woolly lemur, at Fandriana District (Bemosary Classified Forest). *A. betsilio* was formerly referred to as *Avahi* species *nova* #1. Photo by John R. Zaonarivelo.

A comprehensive dataset that includes representatives from multiple populations and sequence fragments for all recognized genera and species will be required to conclusively resolve the taxonomic relationships between the family Indriidae and other genera, but this data does offer corroborative evidence for establishing species versus subspecies status for regional populations of the genus *Avahi* based on the phylogenetic species concept (Cracraft 1983). For instance, Zaramody et al. (2006) suggested that the status of *A. cleesei* was questionable based on a relatively low genetic distance between *A. cleesei* and *A. occidentalis*. By evaluating the uncorrected genetic distance, morphological, phenotypic, and pairwise aggregate analysis data, along with the inclusion of the third recognized western *Avahi*, *A. unicolor*, we conclude that the parameters generated with the multiple data sets are analogous to those established with other species, including comparisons within the genera *Propithecus* and *Lepilemur* (Figs. 8-14; Tables 1-4; Appendix Id; Mayor et al. 2004; Thalmann and Geissmann 2005, 2006; Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b). For example, there exist significant differences within the cytogenetic, phenotypic, and morphological data among sifaka species which consequently led to full species status for seven sifakas of the genus *Propithecus* (Mayor et al. 2004; Mittermeier et al. 2006). Despite the elevation to full species status for the sifakas, the genetic distance based on D-Loop sequence data between *Propithecus tattersalli* and *P. coquereli* or *P. edwardsi* and *P. diadema* is relatively modest (1.79 and 1.96, respectively; Mayor et al. 2004). The minimal genetic distance between individual sifaka species, however, can establish supportive evidence in favor of the legitimacy of *A. cleesei* since the genetic distance between each pair of sifaka mentioned previously is less than estimated between *A. cleesei* and *A. occidentalis* (3.86) and/or *A. unicolor* (4.17; Mayor et al. 2004).

Despite several citations which identify type locality of *Avahi laniger* in the Betanimena country, Antongil Bay, Maroantsetra, the type locality of *A. laniger* remains unknown or uncertain (Jenkins 1987; Groves 2001; Zaramody et al. 2006). Nonetheless, based on phenotypic and molecular data (PAST fragment only), two types of *A. laniger* from the extreme ends of its range are evident within these data (Figs. 1, 4, and 15; Tables 2-4; Mantadia and Mananara-Nord National Parks). Further research, including comprehensive samples and data generation, will be needed, however, to conclusively resolve the taxonomic relationships of the woolly lemurs between the Mangoro/Onive Rivers and Anjanaharibe-Sud Special Reserve.

The data also established two types of *A. peyrierasi* (Figs. 8-14, 16-17; Tables 1-4). In forest fragments south and north of Ranomafana National Park (Tolongoina Classified Forest; Zaramody et al. 2006), unpublished data reveal that both types of *A. peyrierasi* are found within the same habitat (within Vohiparara, Ambatolahy Dimy, and Talatakely, all subpopulations within Ranomafana National Park). However, no behavioral or hierarchal data exists within a population or family group, so further research (mitochondrial DNA sequence and genotype data (pedigree analysis) combined with long term behavioral observations) is required to establish the relationships between these two types of sympatric *Avahi*. Furthermore, we suggest that the two subspecies, *A. meridionalis meridionalis* and *A. m. ramanantsoavana*, described by Zaramody et al. (2006), should be elevated to full species because the molecular, phenotypic, and morphological data is equivalent, if not more conclusive, to the other previously recognized woolly lemurs (Figs. 8-14, 18-22; Tables 1-4).

In this paper, the current *Avahi* taxonomy was examined according to the Phylogenetic Species Concept (PSC) *sensu* Wheeler and Platnick et al. (2000), Mayor et al. (2004), and Louis et al. (2006a, 2006b). The diagnostic characters or attributes define evolutionary significant units (ESUs). Several authors suggest that ESUs are equivalent to species as determined through the Phylogenetic Species Concept (Cracraft 1983; Barrowclough and Flesness 1996; Amato et al. 1998). The constant addition of samples to the PAA data set will continue to test the distinction of these characters. The identification of a new species in the following description establishes the essential need for extensive as well as detailed sample collections across Madagascar to determine geographic ranges and taxonomic status for all of the woolly lemurs. This research is especially needed on opposite aspects of all rivers (for example, north of the Mangoro River to Anjanaharibe-Sud Special Reserve). Due to the inability to maintain woolly lemurs as long-term live vouchers in captivity, whole blood, morphometric, and e-voucher photos will

serve as the type series for the newly described species, *Avahi* species *nova* #1, described below. In this case, an attempt was also made to identify existing museum specimens to represent the type series, but a museum specimen has not been identified from this specific region which could serve as the holotype.

Avahi betsileo, New Species

Type Series.—Whole blood for FAN2.14 (TK 125757), adult male; FAN2.15 (TK 125758), adult female; and FAN2.20 (TK 125759), adult male; are stored and curated at the Museum of Texas Tech University. Individual measurements, e-voucher photos, and collection data are given in *Avahi* Field Data Appendix Ia. FAN2.14, FAN2.15, and FAN2.20 were collected by Richard Randriamampionona, Jean C. Randriamanana, Gerard Nalanirina, Razafindraibe Jean, Rambinintsoa Andriantompohavana, and John R. Zaonarivelo on 13 July 2002, 13 July 2002, and 15 July 2002, respectively.

Type Locality.—Madagascar, Province de Fianarantsoa, Region Amoron'i Mania, District of Fandriana, Bemosary Classified Forest, 20°20′60.1″S, 47°33′36.1″E, and south of Mangoro River.

Description.—When *A. betsileo* is compared to other southeastern woolly lemurs, *Avahi betsileo* (1.05 kg) is approximately the same size as *A. peyrierasi* type #2 (0.86 kg) and *A. meridionalis* (1.06 kg), but slightly larger than *A. peyrierasi* type #3 (0.98 kg) and *A. ramanantsoavana* (0.98 kg; Table 1). Phenotypically, *A. betsileo* differs significantly from other eastern woolly lemurs with a pelage that is primarily light reddish-brown on the body and on the dorsal surface of the extremities (Figs. 23-24). *A. betsileo* has a distinct facial mask with grayish pelage under the mandible and the ventral surface of the extremities. *A. betsileo* has diffuse cream colored eyebrow markings and a thicker pelage on the head which gives a more round or oval-like appearance of the head that distinguishes it from the other eastern woolly lemurs. The venter is dark gray towards the midline and diffuses to a light gray ventrolaterally. The tail is primarily reddish brown, darker on the dorsal surface than the ventral portion which is a lighter reddish blonde.

Diagnosis.—In the D-loop and PAST sequence fragments, *A. betsileo* differs from the closest relatives, *A. peyrierasi* type 2, and *A. peyrierasi* type 3 by 4.75%±0.91% (28 informative sites) - 2.92%±0.37% (68 informative sites) and 3.43%±0.77% (19 informative sites) - 1.33%±0.23% (31 informative sites), respectively. In the D-loop and PAST sequence fragments, *A. betsileo* differs from the closest species relative to geographic distance, *A. laniger*, by 5.50%±0.91% (42 informative sites) and 3.45%±0.37% (99 informative sites), respectively.

Distribution.—*A. betsileo* is currently known in the Bemosary Classified forest (Fandriana) and the regional distribution will be tentatively set from south of the Mangoro River and north of the Mananjary River, until its distribution can be confirmed.

Comparisons and Remarks.—*Avahi betsileo* is approximately the same size as *A. peyrierasi* type #2 and *A. peyrierasi* type #3, larger than *A. ramanantsoavana*, and smaller than *A. laniger* (Table 1). Although *A. betsileo* is not genetically that different from both types of *A. peyrierasi* based on absolute and percentage differences, the phenotypic differences are noticeable (Figs. 23-24). Additional survey work is required to determine the southern and northern range of *A. betsileo* and the northern extent of *A. peyrierasi* type #2 and #3.

Etymology.—The name *betsileo* is proposed for this species and is derived from the Malagasy tribe from the Fandriana region.

Vernacular Name.—Betsileo woolly lemur.

By applying the phylogeographic studies by Thalmann and Geissmann (2000) and Pastorini et al. (2003), geographic barriers can be defined that affect multiple taxa ultimately of both flora and fauna. By establishing and prioritizing these phylogeographic regions, conservation management decisions can be implemented that will support the preservation of biodiversity within these defined regions. These areas of discrete or unique biodiversity show that the all major rivers are significant factors or barriers and augment the processes of speciation (Fig. 25). The emerging data display a distribution of unique biodiversity

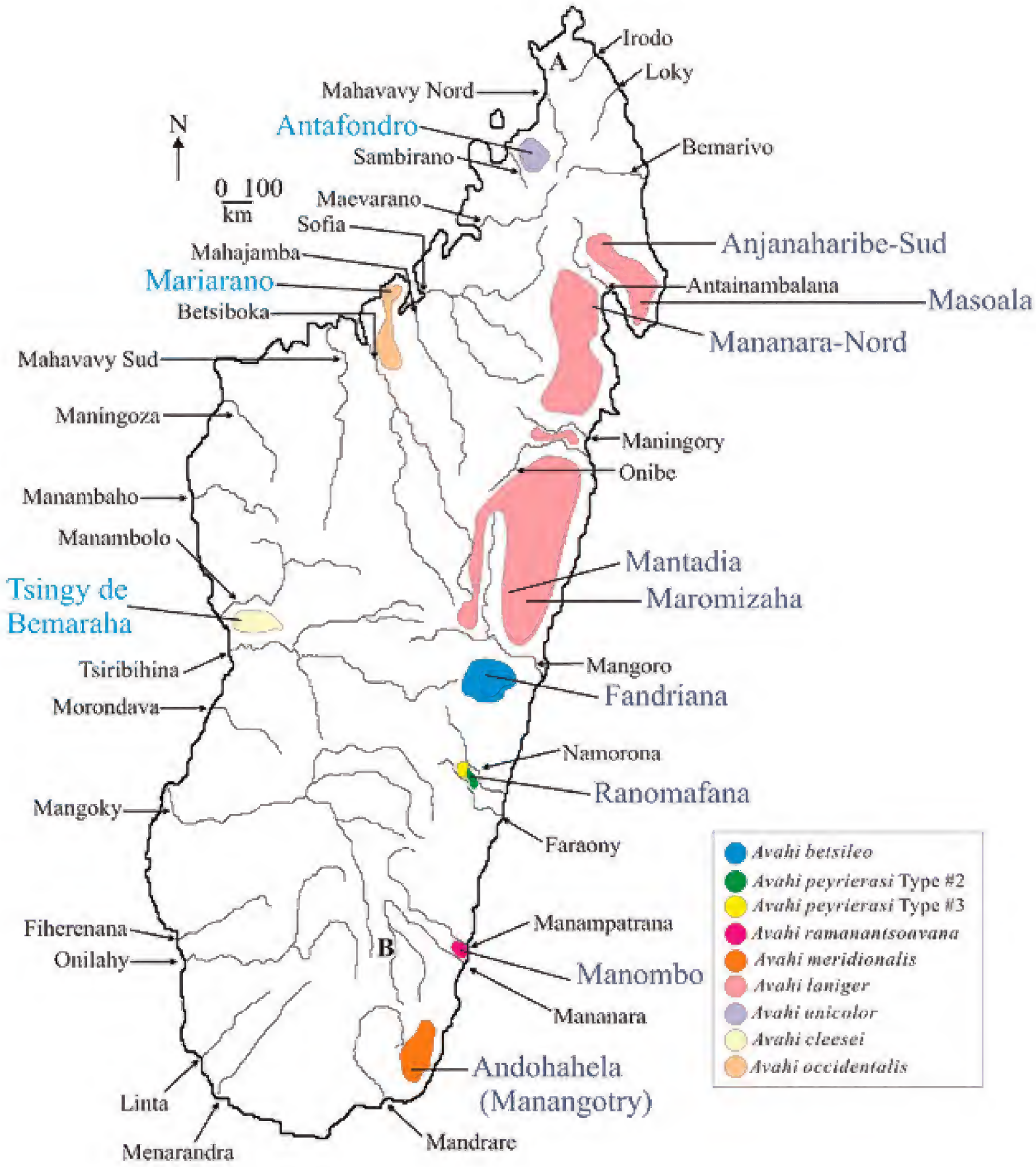

Figure 25. Distribution map of the woolly lemurs of Madagascar - Revised distribution of the woolly lemurs. The letter "A" represents the location of Ankarana Special Reserve. The letter "B" represents the location of Kalambatritra Special Reserve.

framing the topology between all rivers (Andriantompohavana et al. 2006; Louis et al. 2006a, 2006b; Rioux Paquette et al. 2006). This relationship between the rivers and biodiversity transcends taxa and is quite remarkable and consistent (Louis et al. 2006b). As discrete species from different genera are being described, their individual distributions can be overlaid, resulting in river bound "pockets" of unique biodiversity (Cover Figure; unique woolly and sportive lemurs forming paired units of distinct biodiversity). The persistent and rapid loss of habitat and the resulting fragmentation of panmictic populations have compelled wildlife and conservation agencies to take protective action according to existing guidelines and information with the ultimate goal of prioritizing species and/or sites. The explosive rate of the deforestation in Madagascar, however, has eliminated many of the available options (Green and Sussman 1990). Because haplotypes can be unique to each population, simply preserving one population will not necessarily maintain species-wide genetic variability (Pope 1996; Louis et al. 2006b). Despite the recent revisions of the woolly lemurs, isolated fragments or forests that represent unique habitat in Madagascar, such as Kalambatritra and Ankarana Special Reserves and Masoala National Park, could potentially contain particular or distinctive *Avahi* species, so additional comprehensive field work and laboratory work needs to be completed (Cover Figure).

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Addresses of authors:

RAMBININTSOA ANDRIANTOMPOHAVANA

University of Antananarivo
BP 906
Antananarivo 101, Madagascar
E-mail: radambyHDZ@yahoo.fr

RUNHUA LEI

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: leir@omahazoo.com

JOHN R. ZAONARIVELO

University of Antananarivo
BP 906
Antananarivo 101, Madagascar
E-mail: zaonarivelo@yahoo.fr

SHANNON E. ENGBERG

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: genetics@omahazoo.com

GÉRARD NALANIRINA

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: smallvaovao@yahoo.com

SUSIE M. MCGUIRE

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: genetics@omahazoo.com

GARY D. SHORE

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: genetics@omahazoo.com

JUSTIN ANDRIANASOLO

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: smallvaovao@yahoo.com

KELLY HERRINGTON

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: genetics@omahazoo.com

RICK A. BRENNEMAN

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: rabr@omahazoo.com

Addresses of authors (cont.):

EDWARD E. LOUIS, JR.

Center for Conservation and Research
Henry Doorly Zoo
3701 S. 10th St.
Omaha, NE 68107, USA
E-mail: edlo@omahazoo.com
E-mail: (Lab - United States):
genetics@omahazoo.com
Email: (Field - Madagascar):
kelynews1@yahoo.com

APPENDIX I

The following Appendices to this publication are available online at the indicated website addresses.

a. *Avahi* Field Data Appendix (Individual data file for each *Avahi*, including morphometrics, photos, sequence accessions, global position system, microchip data, gender, and location).

http://10.10.10.3/ccr/genetics/lemur/index.asp?page=ccr/genetics/lemur/Avahi.htm

b. Appendix Primer Table I (Summary of designed primers for D-loop and PAST fragments).

http://www.omahazoo.com/ccr/genetics/papers/appendixAvahiprimertableI.pdf

c. Appendix Haplotype Table I (Summary of designated haplotypes for *Avahi* from Fandriana and Ranomafana localities for D-loop and PAST fragments).

http://www.omahazoo.com/ccr/genetics/papers/AvahihaplotypetableI.pdf

d. Appendix ANOVA dendrogram of the morphological data summarized in Table 1.

http://www.omahazoo.com/ccr/genetics/papers/appendixAvahimorphodata.pdf

APPENDIX II

Sample (98 Avahi *and 25 outgroups total) used in the present genetic study and taxonomic revision of the Madagascar lemur genus* Avahi. [a]*TK Number is referenced voucher curated at the Museum of Texas Tech University.* [b]*Mitochondrial DNA sequence data for D-Loop (D-Loop or control region) and PAST (Pastorini fragment) for each sample are available from GenBank under the listed accession number. N/A signifies that sequence fragment is not available. (*A. betsileo *was formerly referred to as* Avahi *species* nova *#1).*

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
RANO66		Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855975	DQ856051
RANO67		Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #3	AY584495	AY582558
RANO199	125789	Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #3	DQ855976	DQ856052
RANO261	125790	Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #3	AY584496	AY582559
RANO319B		Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855977	DQ856053
RANO320B	125791	Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF187751	DQ856054
RANO348		Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855978	DQ856055
RANO2.10	125751	Ranomafana (Talatakely)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855979	DQ856056
RANOL6.1		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491654	EF491638
RANOL6.2		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491655	EF491639
RANOL6.3		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491656	EF491640
RANOL6.4		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491657	EF491641
RANOL6.11		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491658	EF491642

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
RANOL6.12		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491659	EF491643
RANOL6.13		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491660	EF491644
RANOL6.14		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491661	EF491645
RANOL6.15		Ranomafana (Ambatolahy Dimy)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491662	EF491646
RANOV6.7		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491647	EF491631
RANOV6.8		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491648	EF491632
RANOV6.10		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491649	EF491633
RANOV6.11		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	EF491650	EF491634
RANOV6.12		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491651	EF491635
RANOV6.13		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491652	EF491636
RANOV6.22		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #3	EF491653	EF491637
VOP2.13	125752	Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855980	DQ856057
VOP2.14	125753	Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855981	DQ856058
VOP2.15		Ranomafana (Vohiparara)	*Avahi laniger*	*Avahi peyrierasi* type #2	DQ855982	DQ856059
M86	125754	Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855983	DQ856060
M89		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855984	DQ856061

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
M107	125755	Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855985	DQ856062
M108		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855986	DQ856063
M119		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855987	DQ856064
M150		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855988	DQ856065
M155		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855989	DQ856066
M159		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855990	DQ856067
MAB4.8	125756	Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855991	DQ856068
MAB4.10		Manombo	*Avahi laniger*	*Avahi m. ramanantsoavana*	DQ855992	DQ856069
FAN2.14	125757	Fandriana	*Avahi laniger*	*Avahi betsileo*	DQ855993	DQ856070
FAN2.15	125758	Fandriana	*Avahi laniger*	*Avahi betsileo*	DQ855994	DQ856071
FAN2.19		Fandriana	*Avahi laniger*	*Avahi betsileo*	DQ855995	DQ856072
FAN2.20	125759	Fandriana	*Avahi laniger*	*Avahi betsileo*	DQ855996	DQ856074
FAN2.21		Fandriana	*Avahi laniger*	*Avahi betsileo*	DQ855997	DQ856073
FAN6.6		Fandriana	*Avahi laniger*	*Avahi betsileo*	EF396279	EF396276
FAN6.7		Fandriana	*Avahi laniger*	*Avahi betsileo*	EF396280	EF396277
FAN6.9		Fandriana	*Avahi laniger*	*Avahi betsileo*	EF396281	EF396278

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
FAN6.10		Fandriana	*Avahi laniger*	*Avahi betsileo*	EF396282	EF396275
FAN6.11		Fandriana	*Avahi laniger*	*Avahi betsileo*	EF396283	EF396274
TAD1		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ855998	DQ856075
TAD2	125760	Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ855999	DQ856076
TAD3		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856000	DQ856077
TAD19		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856001	DQ856078
TAD36		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856002	DQ856079
TAD37		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856003	DQ856080
TAD4.3	125761	Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856004	DQ856081
TAD4.8		Mantadia	*Avahi laniger*	*Avahi laniger* type #1	DQ856005	DQ856082
TAD4.9	125762	Mantadia NP	*Avahi laniger*	*Avahi laniger* type #1	DQ856006	DQ856083
TAD4.20		Mantadia NP	*Avahi laniger*	*Avahi laniger* type #1	DQ856007	DQ856084
MIZA10		Maromizaha CF	*Avahi laniger*	*Avahi laniger* type #1	DQ856008	DQ856085
MIZA12		Maromizaha CF	*Avahi laniger*	*Avahi laniger* type #1	DQ856009	DQ856086
MIZA13		Maromizaha CF	*Avahi laniger*	*Avahi laniger* type #1	DQ856011	DQ856087
MIZA14		Maromizaha CF	*Avahi laniger*	*Avahi laniger* type #1	DQ856010	DQ856088

APPENDIX II (CONT.)

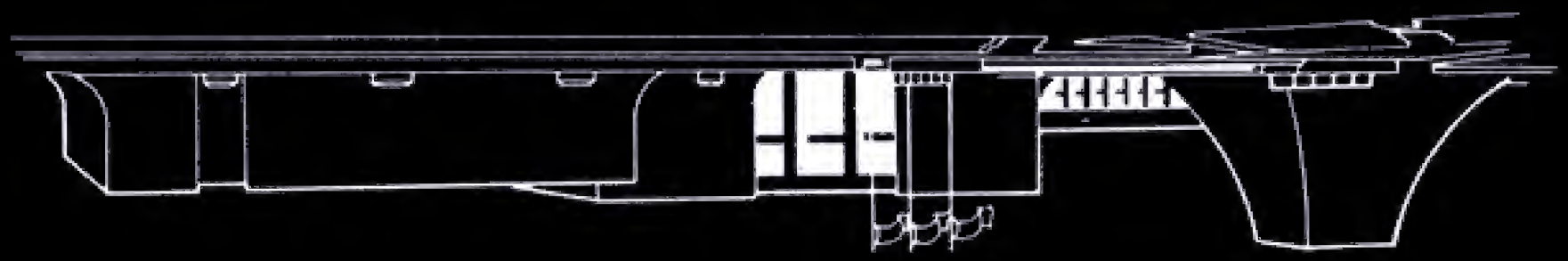
Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
AND4	125763	Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856012	DQ856089
AND5	125764	Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856013	DQ856090
AND6		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856014	DQ856091
AND11		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856015	DQ856092
AND12		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856016	DQ856093
AND13	125765	Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856017	DQ856094
AND14		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856018	DQ856095
AND19		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856019	DQ856096
AND33		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856020	DQ856097
AND34		Andohahela (Manangotry)	*Avahi laniger*	*Avahi m. meridionalis*	DQ856021	DQ856098
NARA4.1		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856022	DQ856099
NARA4.2		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856023	DQ856100
NARA4.10		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856024	DQ856101
NARA4.11	125766	Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856025	DQ856102
NARA4.13		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856026	DQ856103
NARA4.17		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856027	DQ856104

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
NARA4.18	125767	Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856028	DQ856105
NARA4.23		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856029	DQ856106
NARA4.31	125768	Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856030	DQ856107
NARA4.32		Mananara-Nord (Ivontaka-Sud)	*Avahi laniger*	*Avahi laniger* type #2	DQ856031	DQ856108
ANT5.8	125769	Antafondro (Maromiandra)	*Avahi unicolor*	*Avahi unicolor*	DQ856032	DQ856109
ANT5.9	125770	Antafondro (Maromiandra)	*Avahi unicolor*	*Avahi unicolor*	DQ856033	DQ856110
ANT5.10	125771	Antafondro (Maromiandra)	*Avahi unicolor*	*Avahi unicolor*	DQ856034	DQ856111
ANT5.12		Antafondro (Maromiandra)	*Avahi unicolor*	*Avahi unicolor*	DQ856035	DQ856112
BEMA8	125772	Tsingy de Bemaraha	*Avahi cleesei*	*Avahi cleesei*	DQ856036	DQ856113
BEMA9	125773	Tsingy de Bemaraha	*Avahi cleesei*	*Avahi cleesei*	DQ856037	DQ856114
BEMA12	125774	Tsingy de Bemaraha	*Avahi cleesei*	*Avahi cleesei*	DQ856038	DQ856115
BEMA13		Tsingy de Bemaraha	*Avahi cleesei*	*Avahi cleesei*	DQ856039	DQ856116
BEMA14		Tsingy de Bemaraha	*Avahi cleesei*	*Avahi cleesei*	DQ856040	DQ856117
MAR29		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856041	DQ856118
MAR46		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856042	DQ856119
MAR52		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856043	DQ856120

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
MAR54		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856044	DQ856121
MAR55		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856045	DQ856122
MAR56		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856046	DQ856123
MAR60		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856047	DQ856124
MAR61		Mariarano	*Avahi occidentalis*	*Avahi occidentalis*	DQ856048	DQ856125
ANK7		Ankarafantsika	*Microcebus ravelobensis*	*Microcebus ravelobensis*	AY159695	AY582545
RANO250		Ranomafana	*Microcebus rufus*	*Microcebus rufus*	AY159722	AY582546
GAR8		Manongarivo	*Cheirogaleus medius*	*Cheirogaleus medius*	AY584498	AY582562
RANO229		Ranomafana	*Cheirogaleus major*	*Cheirogaleus major*	AY254050	AY582563
FAN21		Fandriana	*Varecia variegata*	*Varecia variegata*	AY584494	AY582555
RANO45		Ranomafana	*Eulemur fulvus rufus*	*Eulemur fulvus rufus*	AY585738	AY582561
ANAL2.23		Analamerana	*Hapalemur g. occidentalis*	*Hapalemur g. occidentalis*	AY584493	AY582554
GAR9		Analamerana	*Hapalemur g. occidentalis*	*Hapalemur g. occidentalis*	AY584492	AY582553
RANO61		Ranomafana	*Hapalemur griseus griseus*	*Hapalemur griseus griseus*	AY584490	AY582551
RANO62		Ranomafana	*Hapalemur griseus griseus*	*Hapalemur griseus griseus*	AY584491	AY582552
KIAN124		Kianjavato	*Hapalemur simus*	*Prolemur simus*	AY584488	AY582548

APPENDIX II (CONT.)

Catalogue Number	TK Number[a]	Location	Original Species Designation	Current Species Designation	D-Loop[b]	PAST[b]
RANO338		Ranomafana	*Hapalemur simus*	*Prolemur simus*	AY254049	AY582547
RANO351		Ranomafana	*Hapalemur aureus*	*Hapalemur aureus*	AY584489	AY582549
RANO352		Ranomafana	*Hapalemur aureus*	*Hapalemur aureus*	AY254048	AY582550
JAR4		Anjanaharibe-Sud	*Indri indri*	*Indri indri*	DQ855969	DQ856049
MIZA5.3		Maromizaha	*Indri indri*	*Indri indri*	DQ855967	DQ856050
JAR14		Anjanaharibe-Sud	*Propithecus candidus*	*Propithecus candidus*	DQ855969	N/A
TAD13		Mantadia	*Propithecus diadema*	*Propithecus diadema*	DQ855970	N/A
RANO332		Ranomafana	*Propithecus edwardsi*	*Propithecus edwardsi*	AY585739	AY582556
ANAL10		Analamerana	*Propithecus perrieri*	*Propithecus perrieri*	DQ855968	N/A
BOR2		Bora	*Propithecus coquereli*	*Propithecus coquereli*	DQ855971	N/A
BEMA4		Tsingy de Bemaraha	*Propithecus deckeni deckeni*	*Propithecus deckeni deckeni*	DQ855973	N/A
JAM4.7		Anjahamena	*Propithecus d. coronatus*	*Propithecus d. coronatus*	DQ855970	N/A
DAR4.2		Daraina	*Propithecus tattersalli*	*Propithecus tattersalli*	DQ855972	N/A
MOR68		Beroboka	*Propithecus verreauxi*	*Propithecus verreauxi*	AF585739	AY582557